Year : 2012

Growth decrease in Alpine whitefish: investigating the relative contribution of fishing induced selection and environmental change and its implication for conservation measures.

Sébastien Nusslé

UNIL | Université de Lausanne
Département d'écologie
et évolution

Growth decrease in Alpine whitefish: investigating the relative contribution of fishing induced selection and environmental change and its implication for conservation measures

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de Biologie et de Médecine de l'Université de Lausanne

par

Sébastien Nusslé

Diplômé de Biologie

Université de Lausanne

Jury

Prof. Dr. Henrik Kaessmann, Président
Prof. Dr. Daniel Cherix, Directeur de Thèse
Dr. Jean-François Rubin, Expert
Prof. Dr. Nicolas Perrin, Expert

Lausanne, 2012

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale

Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président	Monsieur	Prof. Henrik **Kaessmann**
Directeur de thèse	Monsieur	Prof. Daniel **Cherix**
Experts	Monsieur	Prof. Nicolas **Perrin**
	Monsieur	Dr Jean-François **Rubin**

le Conseil de Faculté autorise l'impression de la thèse de

Monsieur Sébastien Nusslé

Biologiste diplômé de l'Université de Lausanne

intitulée

Growth decrease in Alpine whitefish: investigating the relative contribution of fishing induced selection and environmental change and its implication for conservation measures

Lausanne, le 9 novembre 2012

pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Henrik Kaessmann

Man tends to increase at a greater rate than his means of subsistence.

It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.

I have called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection.

Charles Darwin

On est riche de ce qu'on laisse, non de ce qu'on prend

Robert Hainard

A ma femme Semira
Merci pour ton amour et ton soutien

Table of content

Résumé

La pression exercée par les activités humaines menace pratiquement tous les écosystèmes aquatiques du globe. Ainsi, sous l'effet de divers facteurs tels que la pollution, le réchauffement climatique ou encore la pêche industrielle, de nombreuses populations de poissons ont vu leurs effectifs chuter et divers changements morphologiques ont été observés. Dans cette étude, nous nous sommes intéressés à une menace particulière: la sélection induite par la pêche sur la croissance des poissons. En effet, la génétique des populations prédit que la soustraction régulière des individus les plus gros peut entraîner des modifications rapides de certains traits physiques comme la croissance individuelle. Cela a par ailleurs été observé dans de nombreuses populations marines ou lacustres, dont les populations de féras, bondelles et autres corégones des lacs suisses. Toutefois, malgré un nombre croissant d'études décrivant ce phénomène, peu de plans de gestion en tiennent compte, car l'importance des effets génétiques liés à la pêche est le plus souvent négligée par rapport à l'impact des changements environnementaux. Le but premier de cette étude a donc été de quantifier l'importance des facteurs génétiques et environnementaux.

Dans le premier chapitre, nous avons étudié la population de palée du lac de Joux (*Coregonus palaea*). Nous avons déterminé les différentiels de sélection dus à la pêche, c'est-à-dire l'intensité de la sélection sur le taux de croissance, ainsi que les changements nets de croissance au cours du temps. Nous avons observé une baisse marquée de croissance et un différentiel de sélection important indiquant qu'au moins 30% de la diminution de croissance observée était due à la pression de sélection induite par la pêche. Dans le deuxième chapitre, nous avons effectué les mêmes analyses sur deux espèces proches du lac de Brienz (*C. albellus* et *C. fatioi*) et avons observé des effets similaires dont l'intensité était spécifique à chaque espèce. Dans le troisième chapitre, nous avons analysé deux autres espèces : *C. palaea* et *C. confusus* du lac de Bienne, et avons constaté que le lien entre la pression de sélection et la diminution de croissance était influencé par des facteurs environnementaux. Finalement, dans le dernier chapitre, nous avons étudié les effets potentiels de différentes modifications de la taille des mailles des filets utilisés pour la pêche à l'aide de modèles mathématiques.

Nous concluons que la pêche a un effet génétique non négligeable (et donc peu réversible) sur la croissance individuelle dans les populations observée, que cet effet est lié à la compétition pour la nourriture et à la qualité de l'environnement, et que certaines modifications simples de la taille des mailles des filets de pêche pourraient nettement diminuer l'effet de sélection et ainsi ralentir, voir même renverser la diminution de croissance observée.

Summary

Many fish populations have collapsed or are threaten by human activities. One of the potential threats is the selection induced by size-selective fishing, namely fishery-induced evolution. As fishing is usually size-selective it is indeed expected to induce rapid evolutionary changes: individual growth rates for instance are known to have dramatically dropped in many fish populations during the last decades. Despite increasing evidence for fishery-induced evolution, very few management plans take this problem into account because its relative importance as compared to the impact of environmental change is often considered negligible.

In the first chapter, we analyzed the impact of fishing on a population of the Alpine whitefish *Coregonus palaea* in lake Joux. We determined selection differentials linked to fishing, the strength of selection on growth rate and the actual change of growth rate over time. We found a marked decline in growth rate and a significant selection differential for adult growth. We conclude that about 30% of the observed growth decreases might be linked to fishery-induced selection. In the second chapter, we performed the same analyzes on two sympatric species in lake Brienz (*C. albellus* and *C. fatioi)* and found similar result. In addition, we found that the link between selection differentials and phenotypic changes is influenced by species-specific factors.

In the third chapter, we analyzed two additional species in lake Biel (*C. palaea and C. confusus*) that are specialized on different prey sizes and found that these specific factors influencing the link between selection differentials and phenotypic changes might be linked to the environment and to competition for food.

In the last chapter, we studied the potential effects of various regulations with mathematical individual-based models and found that simple modification of the mesh size of the nets used for fishing may prevent fishery-induced evolution of reduced growth.

In conclusion, fishing has a genetic effect on our populations; this effect is linked to competition for food and environment quality; simple regulations may protect these populations.

General Introduction

Humanity has a long history of fishing, starting before the discovery of agriculture with the first human settlements (Butler and O'connor 2004) and becoming intensive in the Middle Ages in Europe (Barrett *et al.* 2004). In 2011, global fishing yields reached 90 million tonnes, with an estimated first-sale value of 91.2 billion US dollars (FAO 2009). The fishing industry provides livelihood and income to 10-12% of the world population, i.e. about 660-820 millions people (FAO 2012) and in 2006, fish provided about 3 billion people with at least 15% of their average per capita animal protein intake (FAO 2009). If marine fisheries have been relatively stable and even decreased recently, the total fishing yield in inland waters have shown a 30% increase since 2004 and are thought to be widely overfished (FAO 2012). Nowadays, this industry is indeed threatened as many fish species are overexploited, and many stocks have collapsed (Zhou *et al.* 2010) and failed to recover even after complete cessation of fishing (Hutchings 2000, 2004; Hutchings and Reynolds 2004; Syrjanen and Valkeajarvi 2010; Salinas *et al.* 2012). The consequences of the collapse of the industry would be dramatic. In Newfoundland, for instance, over 40’000 people lost their jobs following the closure of one cod fishery in 1993 (Hirsch 2002).

One of the major concerns of fishery management is the sustainability of fish stocks as it is now well established that fishing can be harmful for marine populations (Garcia 2012). For instance the depletion of tuna populations has received a lot of attention recently (Cyranoski 2010) and numerous fish species have suffered from rapid phenotypic changes linked to fishing during the last decades (Darimont *et al.* 2009). Overfishing and habitat destruction are obvious causes of population depletion (Botsford *et al.* 1997) and are often compounded by other factors such as pollution

and climate change (Johnson and Welch 2010; Vonlanthen *et al.* 2012; Heath *et al.* 2012). These threats, however, might not be the only ones. In this thesis, we investigate another silent threat that has received increasing attention during the last decade: fishery-induced evolution.

An evolutionary response to systematic fishing is expected for several reasons: *first,* the mortality linked to fishing activities can be very high and often considerably exceeds the natural mortality, with up to 80% of the population harvested (Rijnsdorp 1993; Mertz and Myers 1998; Jackson *et al.* 2001). *Second,* fishing mortality is usually size-selective, typically targeting the largest individuals (Myers and Hoenig 1997; Fukuwaka and Morita 2008). Lastly, size-related traits usually have a genetic basis and significant heritabilities have been reported for many traits (Theriault *et al.* 2007; Carlson and Seamons 2008). Thus, size-selective fishing is expected to induce rapid evolutionary changes (Palumbi 2001; Smith and Bernatchez 2008; Darimont *et al.* 2009) and has therefore been called a "large-scale experiment in life-history evolution" (Rijnsdorp 1993; Law 2000; Stokes and Law 2000; Jensen *et al.* 2012). Life-history traits where fishing is suspected to drive evolutionary change include age or size at maturation (Heino *et al.* 2002; Grift *et al.* 2003; Sharpe and Hendry 2009), average reproductive effort (Yoneda and Wright 2004; Thomas *et al.* 2009), spawning behaviour (Opdal 2010), or individual growth rates (Handford *et al.* 1977; Ricker 1981; Swain *et al.* 2007; Thomas and Eckmann 2007; Nusslé *et al.* 2009; Nusslé *et al.* 2011).

Despite increasing evidence for fishery-induced evolution (Jørgensen *et al.* 2007), including control experiments in the laboratory (Conover and Munch 2002; Walsh *et al.* 2006; Conover and Baumann 2009), there is much discussion regarding the relative importance of fishery-induced evolution as compared to the impact of

phenotypic plasticity in response to environmental change (Hilborn 2006; Browman *et al.* 2008; Smith and Bernatchez 2008; Andersen and Brander 2009). Evolutionary processes are considered relatively slow compared to environmental changes and the importance of genetic changes over conservation-relevant periods of time is often questioned. For instance, rapid changes in phosphorous concentration have been observed in most European lakes over the last decades and these changes are known to have had an impact on growth rates of several fish species (Gerdeaux *et al.* 2006; Müller *et al.* 2007; Thomas and Eckmann 2007; Thomas and Eckmann 2012). Phenotypic plasticity is important in fish (Thorpe 1998; Crozier *et al.* 2008) and environmentally induced changes are often correlated with changes linked to the systematic removal of particular genotypes due to selective fishing (Hutchings and Fraser 2008). Therefore, separating the effects of fishery versus environmentally induced changes on individual growth rates is difficult (Heino *et al.* 2008). A critical step in disentangling these effects is to measure the actual strength of selection linked to fishing gears, namely the selection differential. In association with measures of heritability, selection differential would allow to build prediction on the expected changes that are solely due to genetic factors (Law 2000; Law 2007; Smith and Bernatchez 2008).

Alpine whitefish (*Coregonus* sp., Salmonidae) are ideal models to study the interaction between fishery-induced evolution and environmental changes (Müller *et al.* 2007; Thomas and Eckmann 2007; Nusslé *et al.* 2009; Thomas *et al.* 2009; Nusslé *et al.* 2011) for several reasons. *First,* Alpine whitefish populations may provide relatively accurate measures of selection differentials as migration is almost non-existent and the vast majority of individuals are eventually harvested and do not die of senescence (Müller *et al.* 2007; Nusslé *et al.* 2009; Nusslé *et al.* 2011). *Second,* the

fishing of many Alpine whitefish populations is relatively constant and has been well monitored for several decades. *Third*, there are a large number of different populations, and many lakes even have several sympatric species with little gene flow between them (Douglas *et al.* 1999; Douglas *et al.* 2005; Hudson *et al.* 2007; Vonlanthen *et al.* 2009; Bittner *et al.* 2010). Lastly, these populations have seen different changes in phosphorus concentration, which is known to have a major impact on whitefish populations, since the 80s. Therefore closely-related populations experiencing different well-documented environmental conditions, combined with well-monitored fishing-induced selection pressure is a unique opportunity to study the interaction between environmental changes and fishery-induced genetic changes on life-history traits such as growth rate.

In this thesis we analysed long-term monitoring surveys to determine the selection differentials and the phenotypic changes over several generations of five Alpine whitefish populations inhabiting three different lakes that experienced different phosphorus changes. We found that evolutionary changes are relatively important for these populations and that the link between selection differentials and phenotypic changes might be influenced by the environment and by species-specific characteristics linked to competition for food. Although fishery-induced evolution could dramatically change populations (Fenberg and Roy 2008), so far only few management plans have taken this problem into account (Stokes and Law 2000; Ashley *et al.* 2003; Smith and Bernatchez 2008) and it is still not clear how management policies should be changed to best react to the situation. We therefore used individual-based modelling to predict the evolutionary consequences of different size-selective fishing gear in order to determine some preliminary guidelines for fishery management.

Bibliography

Andersen, K. H., and K. Brander. 2009. Expected rate of fisheries-induced evolution is slow. Proceedings of the National Academy of Sciences of the United States of America **106**:11657-11660.

Ashley, M. V., M. F. Wilson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation **111**:115-123.

Barrett, J. H., A. M. Locker, and C. M. Roberts. 2004. The origins of intensive marine fishing in medieval Europe: the English evidence. Proceedings of the Royal Society of London B Biological Sciences **271**:2417-2421.

Bittner, D., L. Excoffier, and C. R. Largiader. 2010. Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus spp.*) in a Swiss lake: a role for eutrophication? Molecular Ecology **19**:2152-2167.

Botsford, L. W., J. C. Castilla, and C. H. Peterson. 1997. The management of fisheries and marine ecosystems. Science **277**:509-515.

Browman, H. I., R. Law, C. T. Marshall, A. Kuparinen, J. Merila, C. Jørgensen, K. Enberg, *et al.* 2008. The role of fisheries-induced evolution. Science **320**:47-50.

Butler, V. L., and J. E. O'connor. 2004. 9000 years of salmon fishing on the Columbia River, North America. Quaternary Research **62**:1-8.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications **1**:222-238.

Conover, D. O., and H. Baumann. 2009. The role of experiments in understanding fishery-induced evolution. Evolutionary Applications **2**:276-290.

Conover, D. O., and S. B. Munch. 2002. Sustaining fisheries yields over evolutionary time scales. Science **297**:94-96.

Crozier, L. G., A. P. Hendry, P. W. Lawson, T. P. Quinn, N. J. Mantua, J. Battin, R. G. Shaw, *et al.* 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications **1**:252-270.

Cyranoski, D. 2010. Pacific tuna population may crash at any time. Nature **465**:280-281.

Darimont, C. T., S. M. Carlson, M. T. Kinnison, P. C. Paquet, T. E. Reimchen, and C. C. Wilmers. 2009. Human predators outpace other agents of trait change in the wild. Proceedings of the National Academy of Sciences of the United States of America **106**:952-954.

Douglas, M. R., P. C. Brunner, and L. Bernatchez. 1999. Do assemblages of Coregonus (Teleostei : Salmoniformes) in the Central Alpine region of Europe represent species flocks? Molecular Ecology **8**:589-603.

Douglas, M. R., P. C. Brunner, and M. E. Douglas. 2005. Evolutionary homoplasy among species flocks of central alpine Coregonus (Teleostei: Salmoniformes). Copeia:347-358.

FAO. 2009. The state of world fisheries and aquaculture. Rome: Fisheries and Aquaculture Department. Food and Agriculture Organisation of the United Nations.

FAO. 2012. The state of world fisheries and aquaculture. Rome: Fisheries and Aquaculture Department. Food and Agriculture Organisation of the United Nations.

Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209-220.

Fukuwaka, M., and K. Morita. 2008. Increase in maturation size after the closure of a high seas gillnet fishery on hatchery-reared chum salmon *Oncorhynchus keta*. Evolutionary Applications **1**:376-387.

Garcia, S.M., Kolding, J., Rice, J., Rochet, M.-J., Zhou, S., Arimoto, T., Beyer, J.E., Borges, L., Bundy, A., Dunn, D., *et al*. 2012. Reconsidering the Consequences of Selective Fisheries. Science **335**:1045-1047.

Gerdeaux, D., O. Anneville, and D. Hefti. 2006. Fishery changes during re-oligotrophication in 11 peri-alpine Swiss and French lakes over the past 30 years. Acta Oecologica **30**:161-167.

Grift, R. E., A. D. Rijnsdorp, S. Barot, M. Heino, and U. Dieckmann. 2003. Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Marine Ecology-Progress Series **257**:247-257.

Handford, P., G. Bell, and T. Reimchen. 1977. Gillnet fishery considered as an experiment in artificial selection. Journal of the Fisheries Research Board of Canada **34**:954-961.

Heath, M.R., Neat, F.C., Pinnegar, J.K., Reid, D.G., Sims, D.W., and Wright, P.J. 2012. Review of climate change impacts on marine fish and shellfish around the UK and Ireland. Aquatic Conservation: Marine and Freshwater Ecosystems **22**:337-367.

Heino, M., L. Baulier, D. S. Boukal, E. S. Dunlop, S. Eliassen, K. Enberg, C. Jørgensen, *et al.* 2008. Evolution of growth in Gulf of St Lawrence cod? . Proceedings of the Royal Society of London B Biological Sciences **275**:1111-1112.

Heino, M., U. Dieckmann, and O. R. Godo. 2002. Measuring probabilistic reaction norms for age and size at maturation. Evolution **56**:669-678.

Hilborn, R. 2006. Faith-based fisheries. Fisheries **31**:554-555.

Hirsch, T. 2002. Cod's warning from Newfoundland. *BBC news World edition*, 16 december 2002.

Hudson, A. G., P. Vonlanthen, R. Mueller, and O. Seehausen. 2007. Review: The geography of speciation and adaptive radiation in coregonines. Biology and Management of Coregonid Fishes - 2005 **60**:111-146.

Hutchings, J. A. 2000. Collapse and recovery of marine fishes. Nature **406**:882-885.

Hutchings, J. A. 2004. Evolutionary biology - The cod that got away. Nature **428**:899-900.

Hutchings, J. A., and D. J. Fraser. 2008. The nature of fisheries- and farming-induced evolution. Molecular Ecology **17**:294-313.

Hutchings, J. A., and J. D. Reynolds. 2004. Marine fish population collapses: Consequences for recovery and extinction risk. Bioscience **54**:297-309.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, *et al.* 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629-638.

Jensen, O., Branch, T., and Hilborn, R. 2012. Marine fisheries as ecological experiments. Theoretical Ecology **5**:3-22.

Johnson, J. E., and D. J. Welch. 2010. Marine Fisheries Management in a Changing Climate: A Review of Vulnerability and Future Options. Reviews in Fisheries Science **18**:106-124.

Jørgensen, C., K. Enberg, E. S. Dunlop, R. Arlinghaus, D. S. Boukal, K. Brander, B. Ernande, *et al.* 2007. Ecology - Managing evolving fish stocks. Science **318**:1247-1248.

Law, R. 2000. Fishing, selection, and phenotypic evolution. Ices Journal of Marine Science **57**:659-668.

Law, R. 2007. Fisheries-induced evolution: present status and future directions. Marine Ecology-Progress Series **335**:271-277.

Mertz, G., and R. A. Myers. 1998. A simplified formulation for fish production. Canadian Journal of Fisheries and Aquatic Sciences **55**:478-484.

Müller, R., M. Breitenstein, M. M. Bia, C. Rellstab, and A. Kirchhofer. 2007. Bottom-up control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquatic Sciences **69**:271-288.

Myers, R. A., and J. M. Hoenig. 1997. Direct estimates of gear selectivity from multiple tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences **54**:1-9.

Nusslé, S., C. Bornand, and C. Wedekind. 2009. Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. Evolutionary Applications **2**:200-208.

Nusslé, S., A. Bréchon, and C. Wedekind. 2011 Change in individual growth rate and its link to gill-net fishing in two sympatric whitefish species. Evolutionary Ecology **25**:681-693.

Opdal, A. F. 2010. Fisheries change spawning ground distribution of northeast Arctic cod. Biology letters **6**:261-264.

Palumbi, S. R. 2001. Evolution - Humans as the world's greatest evolutionary force. Science **293**:1786-1790.

Ricker, W. E. 1981. Changes in the average size and average age of pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences **38**:1636-1656.

Rijnsdorp, A. 1993. Fisheries as a large-scale experiment on life-history evolution: disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia **96**:391-401.

Salinas, S., K. O. Perez, T. A. Duffy, S. J. Sabatino, L. A. Hice, S. B. Munch, and D. O. Conover. 2012. The response of correlated traits following cessation of fishery-induced selection. Evolutionnary Applications.

Sharpe, D. M. T., and A. P. Hendry. 2009. Life history change in commercially exploited fish stocks: an analysis of trends across studies. Evolutionary Applications **2**:260-275.

Smith, T. B., and L. Bernatchez. 2008. Evolutionary change in human-altered environments. Molecular Ecology **17**:1-8.

Stokes, K., and R. Law. 2000. Fishing as an evolutionary force. Marine Ecology-Progress Series **208**:307-309.

Swain, D. P., A. F. Sinclair, and J. M. Hanson. 2007. Evolutionary response to size-selective mortality in an exploited fish population. Proceedings of the Royal Society of London B Biological Sciences **274**:1015-1022.

Syrjanen, J., and P. Valkeajarvi. 2010. Gillnet fishing drives lake-migrating brown trout to near extinction in the Lake Paijanne region, Finland. Fisheries Management and Ecology **17**:199-208.

Theriault, V., D. Garant, L. Bernatchez, and J. J. Dodson. 2007. Heritability of life-history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). Journal of Evolutionary Biology **20**:2266-2277.

Thomas, G., and R. Eckmann. 2007. The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth - the Lake Constance example revisited. Canadian Journal of Fisheries and Aquatic Sciences **64**:402-410.

Thomas, G., and R. Eckmann. 2012. Reproduction vs. growth : indications for altered energy fluxes in Lake Constance whitefish through size-selective fishery. Advances in Limnology **63**:147–157

Thomas, G., H. Quoss, J. Hartmann, and R. Eckmann. 2009. Human-induced changes in the reproductive traits of Lake Constance common whitefish (*Coregonus lavaretus*). Journal of Evolutionary Biology **22**:88-96.

Thorpe, J. E. 1998. Salmonid life-history evolution as a constraint on marine stock enhancement. Bulletin of Marine Science **62**:465-475.

Vonlanthen, P., D. Roy, A. G. Hudson, C. R. Largiader, D. Bittner, and O. Seehausen. 2009. Divergence along a steep ecological gradient in lake whitefish (Coregonus sp.). Journal of Evolutionary Biology **22**:498-514.

Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A., Muller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiader, C.R., and Seehausen, O. 2012.

Eutrophication causes speciation reversal in whitefish adaptive radiations. Nature **482**:357-362.

Walsh, M. R., S. B. Munch, S. Chiba, and D. O. Conover. 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecology Letters **9**:142-148.

Yoneda, M., and P. J. Wright. 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Marine Ecology-Progress Series **276**:237-248.

Zhou, S. J., A. D. M. Smith, A. E. Punt, A. J. Richardson, M. Gibbs, E. A. Fulton, S. Pascoe, *et al.* 2010. Ecosystem-based fisheries management requires a change to the selective fishing philosophy. Proceedings of the National Academy of Sciences of the United States of America **107**:9485-9489.

Chapter 1

Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate

Sébastien Nusslé, Christophe Bornand, Claus Wedekind

Published in Evolutionary Applications

Authors' contribution

SN and CB gathered the data.
SN analysed the data.
SN, CB and CW discussed the study and the manuscript.
SN and CW wrote the manuscript.

Evolutionary Applications ISSN 1752-4571

ORIGINAL ARTICLE

Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate

Sébastien Nusslé, Christophe N. Bornand and Claus Wedekind

Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Keywords
artificial selection, contemporary evolution, *Coregonus*, salmonid, selection differential.

Correspondence
Sébastien Nusslé, Department of Ecology and Evolution, University of Lausanne, Biophore, 1015 Lausanne, Switzerland. Tel.: +41 21 692 42 49; fax: +41 21 692 41 65; e-mail: sebastien.nussle@unil.ch

Received: 18 August 2008
Accepted: 20 November 2008
First published online: 17 December 2008

doi:10.1111/j.1752-4571.2008.00054.x

Abstract

Size-selective fishing, environmental changes and reproductive strategies are expected to affect life-history traits such as the individual growth rate. The relative contribution of these factors is not clear, particularly whether size-selective fishing can have a substantial impact on the genetics and hence on the evolution of individual growth rates in wild populations. We analysed a 25-year monitoring survey of an isolated population of the Alpine whitefish *Coregonus palaea*. We determined the selection differentials on growth rate, the actual change of growth rate over time and indicators of reproductive strategies that may potentially change over time. The selection differential can be reliably estimated in our study population because almost all the fish are harvested within their first years of life, i.e. few fish escape fishing mortality. We found a marked decline in average adult growth rate over the 25 years and a significant selection differential for adult growth, but no evidence for any linear change in reproductive strategies over time. Assuming that the heritability of growth in this whitefish corresponds to what was found in other salmonids, about a third of the observed decline in growth rate would be linked to fishery-induced evolution. Size-selective fishing seems to affect substantially the genetics of individual growth in our study population.

Introduction

Human activities have caused phenotypic changes in many ecosystems (Palumbi 2001; Smith and Bernatchez 2008). These changes can be rapid, with large modifications occurring within decades only (Thompson 1998; Hendry and Kinnison 1999; Stockwell et al. 2003; Hairston et al. 2005). In many fish populations, for instance, significant shifts in life-history traits have been described. These shifts include maturation at smaller age or size (Heino et al. 2002; Grift et al. 2003), elevated reproductive effort (Yoneda and Wright 2004) and changes in individual growth rate (Handford et al. 1977; Ricker 1981; Thomas and Eckmann 2007). Many of these phenotypic changes may be linked to fishery-induced evolution. In experiments, the systematic removal of larger fish indeed decreases the mean weight of descendants (Conover and Munch 2002) and impacts various life-history traits (Walsh et al. 2006; Hutchings and Rowe 2008). See Jorgensen et al. (2007) for a review on phenotypic traits for which evolutionary changes are likely, and Hard et al. (2008) for a discussion of evolutionary consequences of fishing on salmon.

Several conditions are mandatory for evolution to occur, and fishing on wild populations usually fulfils all these conditions. First, fishing-induced mortality can be very high and may exceed natural mortality by far more than 100% (Rijnsdorp 1993; Mertz and Myers 1998; Jackson et al. 2001). Second, fishing is typically selective with regard to size (Myers and Hoenig 1997; Fukuwaka and Morita 2008). Third, heritable variance has been found for many life-history traits in fish and can be as large as 0.5 (Theriault et al. 2007). Fishing has therefore been called a 'large-scale experiment in life-history evolution' (Rijnsdorp 1993; Law 2000; Stokes and Law 2000).

There is, however, much controversy regarding the relative importance of fishery-induced evolution as compared to the impact of phenotypic plasticity in response to environmental change (Hilborn 2006). It is often questioned whether significant genetic changes over conservation-relevant periods of time are frequent, as discussed in Smith and Bernatchez (2008). Phenotypic plasticity is important in fish (Thorpe 1998; Crozier et al. 2008), and many alleged adaptations could indeed be environmentally induced phenotypic responses rather than genetic changes (Gienapp et al. 2008; Hendry et al. 2008). Changes in eutrophication (Gerdeaux and Perga 2006), salinity (Ricker 1981), temperature (Thresher et al. 2007), competition (Lorenzen and Enberg 2002), or large-scale ocean regime shifts (Pearcy 1992; Thresher et al. 2007) can have an immediate impact on phenotypic traits, particularly life-history traits, without necessarily changing the genetics of a population.

The relative importance of both fishery-induced evolution and phenotypic plasticity is thus a key issue that needs to be addressed (Law 2000, 2007; Smith and Bernatchez 2008). To date, only few studies have tried to separate the effects of fishery-induced evolution and environment-induced changes on individual growth rate. A major problem in such studies is that additive genetic effects can be correlated with long-term changes in, for example, population density, water temperature, or phosphorus concentration and hence productivity of typical freshwater habitats (Hutchings and Fraser 2008). Classical statistical tools such as multiple regressions can therefore be problematic. Recently Swain et al. (2007) used back-calculated length at age 4 (from otolith measurements) to determine the difference in growth between parental and offspring generations of Atlantic cod (*Gadus morhua*) in the Gulf of St.-Lawrence. The authors found significant length at age differences between the generations and concluded that these differences indicate genetic change in growth, i.e. that they may reveal genetic effects of size-selective fishing. Heino et al. (2008) discuss several potential limitations to Swain et al.'s approach, especially that their approach did not account for potential changes in reproductive strategies [see Swain et al. (2008) for a further discussion]. Growth rate is indeed influenced by at least three different life-history traits (Heino et al. 2008): (i) growth capacity, i.e. the ability of fish to transform energy intake into body mass, (ii) the maturation schedule, and (iii) the reproductive investment, i.e. the ratio of gonad mass to somatic mass. In the case of selection against fast growers, resource reallocation from growth capacity to reproductive investment is likely (Gadgil and Bossert 1970; Heino et al. 2008), and any observed change in growth rate could therefore be linked to a change in reproductive investment or in maturation schedule rather than, or in addition to, selection against fast growers.

In this study, we applied the method of Swain et al. (2007) to estimate selection differential in a population of the Alpine whitefish *Coregonus palaea*, Fatio 1890 (a freshwater salmonid). We also determined potential indices of resource reallocation over an observational period of 25 years, and we used a growth metric that takes the whole lifespan of the fish into account (i.e. the whole period under fishing-induced selection). Our study population lives in a small and shallow lake. The population is isolated, i.e. no fish from other populations have been introduced into the lake during the observational period, and migration, which can also potentially affect the estimation of the selection differentials, is impossible. Fishing mortality is very high, i.e. most fish are harvested in their first years of life and old individuals are scarce (95% of the fish where caught before the age of 8 years, whereas the oldest individual in the sample was 13+). Moreover, fishing effort can be considered constant and uniform over the study period: for the duration of the study, two fishermen have been harvesting, following regulations that have not significantly changed since 1960. Fisheries data on yield since the late fifties do not show any directional trend with regard to the total whitefish yield. We can thus assume a relatively constant selection differential over several whitefish generations. This is consistent with our estimates of the selection differentials (see Results). Heritability of growth has been studied in various other salmonids and found to be significant (Theriault et al. 2007; Carlson and Seamons 2008). The specific situation of our study population therefore allows us to estimate the evolutionary consequences of fishing-induced selection on growth within the range of the existing heritability estimates.

Methods

The study population is confined to the Lake Joux, Switzerland (lat = 46.63°N, long = 6.28°E, 9.5 km^2, maximum depth = 32 m). In the course of a monitoring programme that started in 1980, a total of 1654 fish were sampled from large catches (on average 75 fish caught each year, ±37 SD). Mean age of the sampled fish was 4.7 years (±1.4 SD). Fish were sampled each year except between 1997 and 2002 when fishing occurred but no monitoring was done. The catches were taken during the spawning season (November and December) at the spawning site with nylon gill nets of 40, 45 and 50 mm mesh size. The total number of eggs that were collected for supplementary breeding was recorded every year. For size measurement, males and females were pooled because no sexual size dimorphism seems to exist in this species.

Total body length was measured in millimetres and scales were taken from above the lateral line between the dorsal and adipose fins for subsequent age determination and back-calculation of previous body lengths. On 719 fish, scale radius and annulus radii, i.e. the distances from the nucleus to the subsequent annuli, were measured using an ocular micrometre for length measurements. Probably due to the high altitude of Lake Joux and the marked temperature differences between summer and winter, annuli on scales are pronounced and allow for easy estimates of fish age and annuli lengths. We back-calculated the length at previous ages of each fish according to the method of Finstad (2003). This method is based on a multiple regression of fish scale including the age and length of the fish. We used a logarithm transformation of fish length and annulus length. From the resulting length-at-age back-calculations, we computed the following two-parameter logarithmic growth curve for each fish:

$$L_i(t) = \alpha_{0i} + \alpha_{ti}\log(t),$$

where $L_i(t)$ is the back-calculated length of each fish at age t, α_{0i} the back-calculated length at age 1, and α_{ti} the logarithmic growth of each fish. Parameter α_{ti} represents the length increase per time unit on a logarithmic scale. We estimated the parameters α_{0i} and α_{ti} for each fish from the back-calculated lengths:

$$\alpha_{ti} = \left(\frac{1}{T_i - 1}\right)\sum_{t=2}^{T_i}\frac{L_i(t) - \alpha_{0i}}{\log(t)},$$

where T_i is the age of each fish at capture.

For each fish, we calculated the length-at-age with two different methods: with the back-calculations and with our logarithmic model. We then assessed the goodness-of-fit of our growth model with an analysis of variance of all the back-calculated lengths as dependant variable and the theoretical lengths fitted with the two-parameter logarithmic model as independent variable (ANOVA: d.f. = 3369, $r^2 = 0.98$, $P < 0.0001$). This model has several advantages: first, it has fewer parameters than other growth models such as a three-parameter von Bertalanffy model. Second, the interpretation of the two parameters is very intuitive: α_0 represents the length at age 1 (i.e. can be understood as juvenile growth) and α_t represents growth after age 1, i.e. approximates adult growth. Third, all the sampled fish are taken into account. With a single size-at-age measure as used in Swain et al. (2007), all the fish younger than the reference age are discarded from the analysis. This can result in a biased estimation of selection differentials that is linked to size-selective fishing, especially if growth varies among cohorts. Moreover a single size-at-age measure is more subject to environmental influence in particular years. This problem is probably less significant in our model as the parameter α_t takes into account growth over several years, i.e. we expect the variance in our growth measure to be smaller than with a single size-at-age measure. Finally it has been shown for Arctic charr (*Salvelinus alpinus*), a freshwater salmonid, that a two-parameter log-linear growth model provides a fit that is at least as good as the von Bertalanffy growth model (Rubin and Perrin 1990).

To detect a potential change in growth parameters over time, α_0 and α_t were averaged for each cohort and a linear regression was calculated. Average growth parameters were the dependant variables and the birth year of the cohort the independent variable. We were interested in the relative growth difference (in %) between two generations and therefore calculated the average relative change over the generations as the observed change in both parameters divided by the average growth parameter and multiplied by the generation time. The average generation time over the generations was estimated according to Stearns (1992) and was calculated over the whole sample for simplicity, without taking the cohorts into account:

$$\text{Generation time} = \frac{\sum_x x l_x m_x}{\sum_x l_x m_x},$$

where x is the age class of the fish, l_x the probability of survival to age x, and m_x the fecundity of age class x. Fecundity was estimated as the probability (P) of being mature at age x times the mean length (L) of the fish in the age class x cubed ($m_x = PL^3$), assuming that fecundity is proportional to the length cubed of the fish (Clark and Bernard 1992).

As a potential indicator for resource reallocation from growth to reproduction, we estimated the average reproductive investment of all the females captured during the spawning season as the proportional volume of egg per female. All females were captured on spawning grounds and were therefore mature. Some females had already partially spawned, i.e. our measure of reproductive investment underestimates the total eggs production. However the magnitude of this error is not likely to change over time. We used the average volume of eggs per reproducing female of each spawning season and divided this value by the mean length cubed of the fish [the allometric relationship between weight and length was: weight = exp(−12.04 + 3.06*ln(length))]. We also estimated the age at maturation for each fish according to Rijnsdorp and Storbeck (1995). This method assumes that growth, i.e. the yearly size increment, is maximal and linear when the fish is immature and decreases after the fish becomes mature because some resources are invested into reproduction instead of growth. We therefore interpret the gap between large and small yearly length increases as

the timing of maturation. We used linear regressions to test for linear trends over time in these two measures.

The expected response to selection (R), i.e. the change in growth rate expected if only selection by fishing is occurring under a constant environment, was estimated from the breeder's equation (Falconer and Mackay 1996):

$$R = h^2 s,$$

where h^2 is the heritability of growth traits and s the selection differential, i.e. the mean difference of a trait between the actual reproducers (the fish surviving to reproduction), and the whole population. Heritability estimates for growth rate in fish range approximately from 0.1 to 0.5 (Law 2000; Garcia de Leaniz et al. 2007; Swain et al. 2007; Theriault et al. 2007; Carlson and Seamons 2008). In this study, we used an intermediate heritability ($h^2 = 0.3$) and two extreme ones ($h^2 = 0.1$ and $h^2 = 0.5$).

The selection differential s was determined for each age class within each cohort by comparing the reproducers, i.e. the fish caught in subsequent years and at older age, with all the fish of that particular age class. We then estimated the average selection differential in every single cohort born in year k, $\overline{s_k}$, as the mean of the selection differentials calculated for each age class. This mean took into account the number of fish in each age class and the relative contribution of each age class to reproduction, i.e. the average fecundity of the age class.

$$\overline{s_k} = \sum_{j=2}^{j_{\max}} p_{jk} w_j s_{jk},$$

where p_{jk} is the proportion of fish born in year k reproducing at age j and w_j is a weighting parameter for each age class j. To account for the differential contribution of each age class within each cohort due to differences in fecundity, the weighting parameter was set as the cube of the average length of the fish within the age class. s_{jk} is the selection differential for age class j within cohort k and is estimated as:

$$s_{jk} = \frac{\left(\frac{1}{n_{jk}} \sum_{i} \sum_{j=2}^{j_{\max}} m r g_{jk}\right) - \overline{g_k}}{\overline{g_k}},$$

where n_{jk} is the number of mature fish born in year k reproducing at age j, m is the maturation status and equals 1 if $j \geq$ age at maturation and 0 otherwise, r is the reproductive status and equals 1 if age at maturation $< j \leq$ age at capture, and 0 otherwise, and $\overline{g_k}$ is the average growth parameter (α_0 or α_t) of all the fish born in year k. The size selectivity of nonfishing induced mortality was considered negligible compared to fishing selection for the estimation of selection differential.

To disentangle the change in growth due to fishery-induced selection from the change due to environmental variation, we postulated that both factors were additive, i.e. that the observed growth change was equal to the sum of genetic (estimated by $h^2 s$) and phenotypic plasticity. To simplify, we do not take into account a potential interaction between environment and genotype. The fraction of change due to fishery-induced selection was finally calculated as $h^2 s$ divided by the total observed change in growth.

All analyses were carried out on the open-access statistical software 'R' (R Development Core Team 2008). Population means are presented as mean ± standard deviation. All P-values are two-tailed.

Results

We did not observe any significant linear change in resource reallocation from growth to reproduction over the observational period. Neither the maturation schedule, estimated by the mean age at maturation, nor the fecundity, estimated by the proportional volume of eggs per fish, seems to change consistently over time (maturation schedule: $t_{21} = -0.08$, $P = 0.94$, Fig. 1A; fecundity: $t_{16} = 1.32$, $P = 0.20$, Fig. 1B; the years 2000 and 2001 cannot be considered outliers in Fig. 1A as tested with Cook's distances). The potential periodicity observed in fecundity (Fig. 1B) may be linked to intra-specific competition between age classes (Naceur and Büttiker 1999).

Length at age 1 (α_0) did not linearly change over time ($t_{18} = -0.34$, $P = 0.74$, Fig. 2A). However, logarithmic growth (α_t) declined by $-0.94 \pm 0.36\%$ per year ($t_{18} = -2.6$, $P = 0.017$, Fig. 2B). The average generation time, i.e. the average age difference between parents and offspring was estimated to be 4.67 years. The relative growth change per generation is then $-4.37 \pm 1.66\%$.

Selection differentials on parameter α_0, i.e. the difference in growth between reproducers and the whole population, did not change linearly over time (linear regression: $t_{21} = 0.50$, $P = 0.62$), neither did the selection differentials on parameter α_t (linear regression: $t_{21} = 1.02$, $P = 0.32$). Moreover, no clear trend was found with these parameters. We therefore considered each s_k as an independent estimation of an average selection differential s over the whole period with a precision that depends on the number of fish on which the estimation is based. As the number of observations per cohort varied, a weighted t-test, with a weighting proportional to the number of fish in each cohort, was used to test whether s was significantly different from zero.

The selection differential for length at age 1 (α_0) was not significantly different from zero ($t_{22} = -0.87$, $P = 0.39$, Fig. 3A). However, the selection differential for

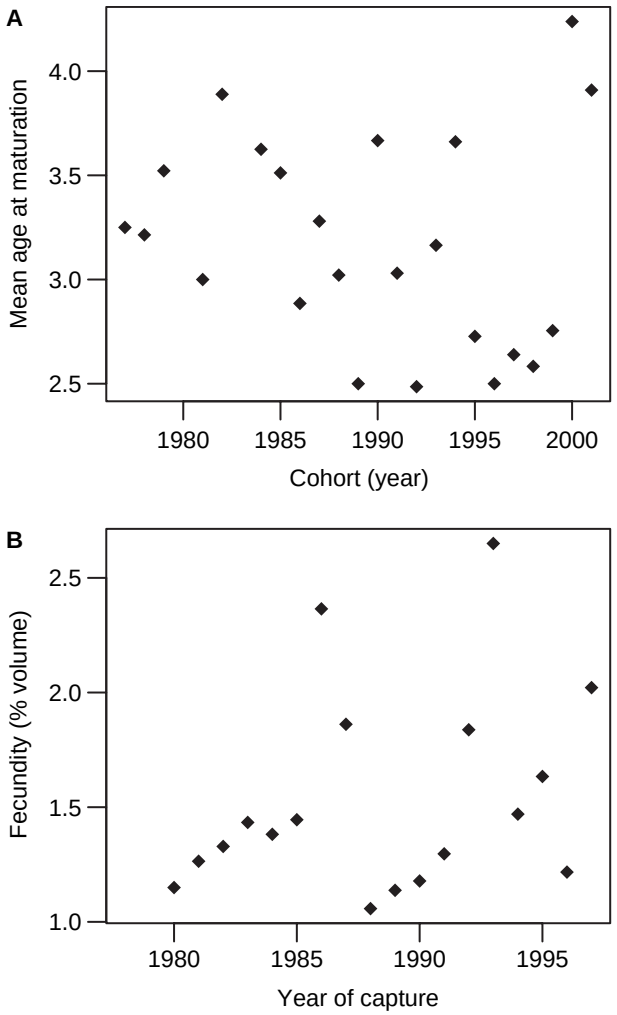


Figure 1 Indicators of resource reallocation from growth to reproduction: (A) reproduction schedule as the mean age at maturation for each cohort and (B) reproductive investment as the mean proportion (in %) of egg volume per female each year of capture during spawning season.

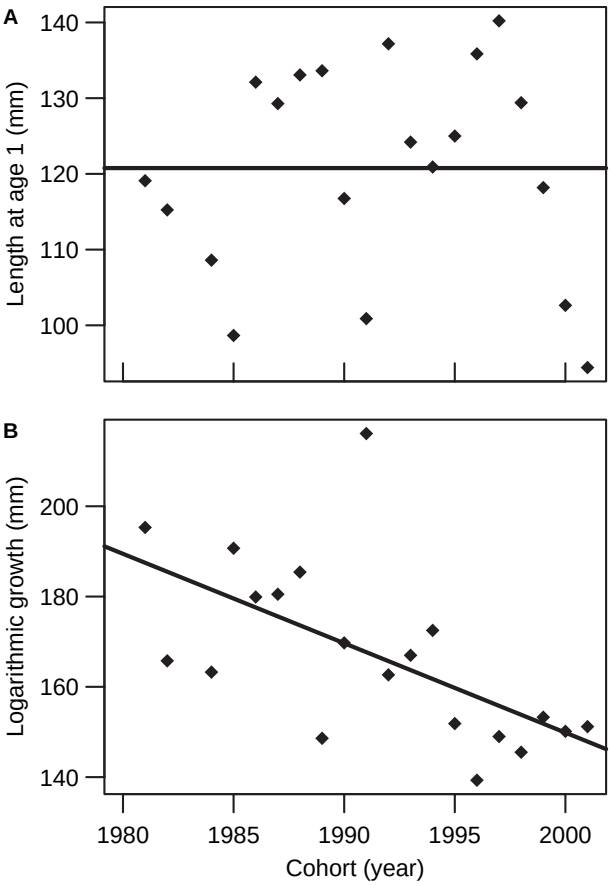


Figure 2 Growth parameters over time: (A) average length at age 1 (α_0) and (B) average logarithmic growth (α_t). The cohort is specified by the year of birth. The lines give the regressions.

the logarithmic growth (α_t) was significantly negative: $s = -4.93 \pm 1.23\%$ ($t_{22} = -4.02$, $P < 0.001$, Fig. 3B).

Assuming that the heritability of growth is $h^2 = 0.3$, with $R = -4.37 \pm 1.67\%$, and $s = -4.93 \pm 1.23\%$, the proportion of logarithmic growth decrease (α_t) due to fishery-induced selection was estimated to be 33.8%. With the two extreme scenarios (i.e. heritability $h^2 = 0.1$ and 0.5), this proportion would be 11.3% and 56.2% respectively.

Discussion

We studied a salmonid population that has been monitored for 25 years. The population is closed to migration and under a fishing pressure that can be considered constant over the observational period. The fishing pressure is strong and most fish that reach maturity seem to be eventually harvested. We therefore consider this population ideal for testing the potential effects of fishery-induced evolution of individual growth rates, a topic that has received much attention recently. We described individual growth with the two parameters α_0 and α_t. The first parameter α_0 describes juvenile growth in the first year of life when the fish are under no direct fishing pressure, whereas the second parameter α_t describes the growth trajectories at later ages and at times when selection by fishing is relevant. We found no evidence that α_0 changed over the last 25 years. However α_t declined significantly during this time.

Changes in individual growth rates over time can be due to fishing-induced evolution, to ecological changes (e.g. temperature, water phosphorus concentration, population density), to a change in life history such as reallocation of resources from growth to reproduction, or to any combination of these possible causes (Heino et al. 2008). Obviously, any increase in energy allocation to

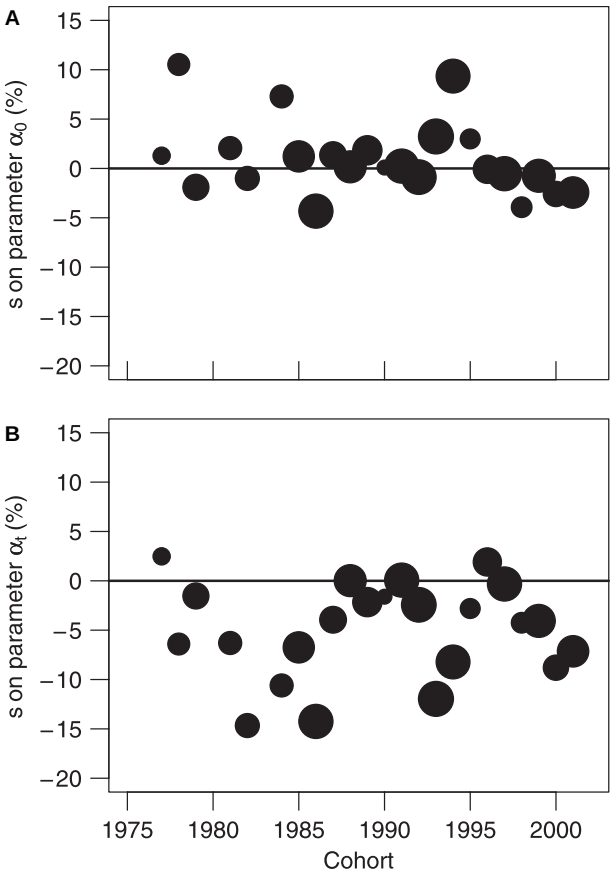


Figure 3 Selection differentials (*s*) estimated for each cohort. (A) *s* for length at age 1 (α_0), and (B) *s* for logarithmic growth (α_t). The width of the circle corresponds to the number of fish within each cohort.

reproduction is expected to slow down growth (Heino and Kaitala 1999). A change in the timing of maturation or in fecundity will therefore change individual growth rates (Stearns 1992). However, we found no evidence for a change in maturation schedule or reproductive strategies in our study population. We therefore concentrate our discussion on the importance of fishing-induced evolution relative to ecological changes over time.

To study fishing-induced evolution, we need to understand the selection induced by fishing, i.e. we need good estimates of the selection differentials. Selection differentials measure the difference in a phenotypic trait between the mean of a population and the mean of the individuals selected to be parents of the next generation. Such phenotypic differences are expected to have a strong genetic component if the fish share the same environmental history. Immigrating individuals and fish escaping harvesting, both common in marine populations, could bias the estimation of selection differentials. In our study population, however, there is no migration and few fish escape eventual harvesting. This, combined with a constant fishing pressure (see Introduction), allows us to determine the selection differentials probably more accurately than analogous determinations in open marine populations. We found that the change in α_t (the growth trajectories at later ages) is around 1% per year or about 4% per generation, but there was no significant change in α_0 (juvenile growth in the first year of life).

Our analyses are however simplifications in several respects. First, we assumed that genetic and ecological factors have additive effects on individual growth rates and that genotype–environment interactions are negligible. Second, we did not apply nonlinear models because of lack of statistical power. Although we have data of >20 cohorts, only about 75 fish were sampled per cohort, and direct parent–offspring comparisons were not possible like in, for example, Grant and Grant (1995) who studied micro-evolutionary responses to directional selection by sampling and assigning parentage to each individual of a population. However, we believe that our model assumptions still lead to useful results in our case because the selection differential can be assumed to vary around an average that does not change over time (the fishing pressure and the reproductive strategies did not seem to change), and nonlinear responses to selection would therefore be somewhat surprising. These assumptions are supported by our data (see Fig. 2).

The evolutionary change in α_t that we observed may be somewhat underestimated because slow growers are more likely to reproduce and die before being caught, i.e. natural mortality may be inversely proportional to size (Conover 2007) and slow growing fish are harvested at an older age because fishing targets fish above a certain size. If we assume that the heritability of growth rates in our study population is about the average of what has been described for salmonids so far (i.e. $h^2 = 0.3$), we conclude that about a third of the decrease in α_t is directly linked to fishing-induced genetic changes in the population. The systematic removal of larger and older fish therefore seems to significantly affect the evolution of individual growth rates in the whitefish of Lake Joux.

The fact that no growth decrease could be observed in juveniles may be surprising. Although there is no fishing pressure on small fish, juvenile and adult growth are likely to be genetically correlated (Lande and Arnold 1983; Walsh et al. 2006). Moreover, everything being equal, juveniles fish that are small may attain a lesser size than large ones and may therefore be likely to suffer less from fishing selection. A possible reason for the observed absence of a decrease might be that α_0 is a single length-at-age measure and therefore more strongly influenced by environmental factors than adult growth (α_t) that is an average over several years. A possible genetic decrease

could therefore be masked by a plastic response to a changing environment. Temperature is known to have a significant impact on juvenile growth (Malzahn et al. 2003; Coleman and Fausch 2007; Gunther et al. 2007). Competition between juveniles and adult may also change with changing average adult size. Finally, there could also be an adaptive response linked to resources reallocation, with more energy invested for juvenile growth to increase juvenile survival and less in adult growth, the *status quo* in juvenile growth could be the maximal viable growth.

The marked decrease in the growth parameter α_t could potentially have negative consequences for the population. There is now mounting evidence that artificial selection such as size-selective harvesting reduces the average viability in some populations (Fenberg and Roy 2008). Several specific consequences may arise from the removal of large fish, and even if these issues are controversial (Carlson et al. 2008), a precautionary approach should be taken when managing evolving fish stocks (Francis and Shotton 1997). First, large and fast-growing individuals may be of higher genetic quality than small and slow-growing individuals (Birkeland and Dayton 2005). A systematic removal of high quality adults could therefore result in an increase of the average genetic load in a population. Second, as large females usually produce larger offspring of higher viability (Trippel 1995; Walsh et al. 2006), a decrease in growth could impair the recruitment and consequently the long-term yield of the population. Third, as females in some species prefer to mate with large males (Hutchings and Rowe 2008), increased mortality of large fish could have an impact on sexual selection and therefore on mating behaviour. Fourth, nonrandom mortality could decrease the genetic diversity of the population and make it more vulnerable to environmental changes or diseases (Jones et al. 2001).

To conclude, we found that the large selection differentials imposed by size-selective fishing can significantly change the genetics of a population. Our data suggest that fishery-induced evolution can be rapid. This needs to be taken into account by population managers (Stokes and Law 2000; Ashley et al. 2003; Smith and Bernatchez 2008).

Acknowledgements

We thank the *Service de la Faune et de Forêts* and the *Service des Eaux, Sols et Assainissement* for providing the monitoring data, the Swiss National Science Foundation for funding, and D. Boukal, S. Cotton, G. Evanno, S. Guduff, A. Jacob, N. Perrin, B. von Siebenthal, T. Szekely Jr, D. Urbach, L. Bernatchez and two anonymous referees for discussion or constructive comments on the manuscript.

Literature cited

Ashley, M. V., M. F. Wilson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation **111**:115–123.

Birkeland, C., and P. K. Dayton. 2005. The importance in fishery management of leaving the big ones. Trends in Ecology & Evolution **20**:356–358.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications **1**:222–238.

Carlson, S. M., E. M. Olsen, and L. A. Vollestad. 2008. Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. Functional Ecology **22**:663–673.

Clark, J. H., and D. R. Bernard. 1992. Fecundity of humpback whitefish and least cisco in the Chatanika River, Alaska. Transactions of the American Fisheries Society **121**:268–273.

Coleman, M. A., and K. D. Fausch. 2007. Cold summer temperature regimes cause a recruitment bottleneck in age-0 Colorado River cutthroat trout reared in laboratory streams. Transactions of the American Fisheries Society **136**:639–654.

Conover, D. O. 2007. Fisheries – nets versus nature. Nature **450**:179–180.

Conover, D. O., and S. B. Munch. 2002. Sustaining fisheries yields over evolutionary time scales. Science **297**:94–96.

Crozier, L. G., A. P. Hendry, P. W. Lawson, T. P. Quinn, N. J. Mantua, J. Battin, R. G. Shaw *et al.* 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications **1**:252–270.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. Benjamin Cummings, Essex, UK.

Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209–220.

Finstad, A. G. 2003. Growth backcalculations based on otoliths incorporating an age effect: adding an interaction term. Journal of Fish Biology **62**:1222–1225.

Francis, R. I. C. C., and R. Shotton. 1997. ''Risk'' in fisheries management: a review. Canadian Journal of Fisheries and Aquatic Sciences **54**:1699–1715.

Fukuwaka, M.-A., and K. Morita. 2008. Increase in maturation size after the closure of a high seas gillnet fishery on hatchery-reared chum salmon *Oncorhynchus keta*. Evolutionary Applications **1**:376–387.

Gadgil, M., and W. H. Bossert. 1970. Life historical consequences of natural selection. The American Naturalist **104**:1.

Garcia de Leaniz, C., I. A. Fleming, S. Einum, E. Verspoor, W. C. Jordan, S. Consuegra, N. Aubin-Horth *et al.* 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews **82**:173–211.

Gerdeaux, D., and M.-E. Perga. 2006. Changes in whitefish scales delta C-13 during eutrophication and reoligotrophication of subalpine lakes. Limnology and Oceanography **51**:772–780.

Gienapp, P., C. Teplitsky, J. S. Alho, J. A. Mills, and J. Merila. 2008. Climate change and evolution: disentangling environmental and genetic responses. Molecular Ecology **17**:167–178.

Grant, P. R., and B. R. Grant. 1995. Predicting microevolutionary responses to directional selection on heritable variation. Evolution **49**:241–251.

Grift, R. E., A. D. Rijnsdorp, S. Barot, M. Heino, and U. Dieckmann. 2003. Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Marine Ecology Progress Series **257**:247–257.

Gunther, S. J., R. D. Moccia, and D. P. Bureau. 2007. Patterns of growth and nutrient deposition in lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*) and their hybrid, F-1 splake (*Salvelinus namaycush* × *Salvelinus fontinalis*) as a function of water temperature. Aquaculture Nutrition **13**:230–239.

Hairston, N. G. J., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters **8**:1114–1127.

Handford, P., G. Bell, and T. Reimchen. 1977. Gillnet fishery considered as an experiment in artificial selection. Journal of the Fisheries Research Board of Canada **34**:954–961.

Hard, J. J., M. R. Gross, M. Heino, R. Hilborn, R. G. Kope, R. Law, and J. D. Reynolds. 2008. Evolutionary consequences of fishing and their implications for salmon. Evolutionary Applications **1**:388–408.

Heino, M., and V. Kaitala. 1999. Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. Journal of Evolutionary Biology **12**:423–429.

Heino, M., U. Dieckmann, and O. R. Godo. 2002. Estimating reaction norms for age and size at maturation with reconstructed immature size distributions: a new technique illustrated by application to Northeast Arctic cod. ICES Journal of Marine Science **59**:562–575.

Heino, M., L. Baulier, D. S. Boukal, E. S. Dunlop, S. Eliassen, K. Enberg, C. Jorgensen *et al.* 2008. Evolution of growth in Gulf of St Lawrence cod? Proceedings of the Royal Society B: Biological Sciences **275**:1111–1112.

Hendry, A. P., and M. T. Kinnison. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. International Journal of Organic Evolution **53**:1637–1654.

Hendry, A. P., T. J. Farrugia, and M. T. Kinnison. 2008. Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology **17**:20–29.

Hilborn, R. 2006. Faith-based fisheries. Fisheries **31**:554–555.

Hutchings, J. A., and D. J. Fraser. 2008. The nature of fisheries- and farming-induced evolution. Molecular Ecology **17**:294–313.

Hutchings, J. A., and S. Rowe. 2008. Consequences of sexual selection for fisheries-induced evolution: an exploratory analysis. Evolutionary Applications **1**:129–136.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury *et al.* 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629–638.

Jones, M. W., T. L. McParland, J. A. Hutchings, and R. G. Danzmann. 2001. Low genetic variability in lake populations of brook trout (*Salvelinus fontinalis*): a consequence of exploitation? Conservation Genetics **2**:245–256.

Jorgensen, C., K. Enberg, E. S. Dunlop, R. Arlinghaus, D. S. - Boukal, K. Brander, B. Ernande *et al.* 2007. Ecology – managing evolving fish stocks. Science **318**:1247–1248.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210–1226.

Law, R. 2000. Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science **57**:659–668.

Law, R. 2007. Fisheries-induced evolution: present status and future directions. Marine Ecology Progress Series **335**:271–277.

Lorenzen, K., and K. Enberg. 2002. Density-dependent growth as a key mechanism in the regulation of fish populations: evidence from among-population comparisons. Proceedings of the Royal Society of London Series B: Biological Sciences **269**:49–54.

Malzahn, A. M., C. Clemmesen, and H. Rosenthal. 2003. Temperature effects on growth and nucleic acids in laboratory-reared larval coregonid fish. Marine Ecology Progress Series **259**:285–293.

Mertz, G., and R. A. Myers. 1998. A simplified formulation for fish production. Canadian Journal of Fisheries and Aquatic Sciences **55**:478–484.

Myers, R. A., and J. M. Hoenig. 1997. Direct estimates of gear selectivity from multiple tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences **54**:1–9.

Naceur, N., and B. Büttiker. 1999. La palée du lac de Joux: statistiques de pêche des reproducteurs; âge, croissance et fécondité. Bulletin de la Société Vaudoise des Sciences Naturelles **86**:273–296.

Palumbi, S. R. 2001. Evolution – humans as the world's greatest evolutionary force. Science **293**:1786–1790.

Pearcy, W. G. 1992. Ocean Ecology of North Pacific Salmonids. Washington Sea Grant Program: Distributed by the University of Washington Press, Seattle.

R Development Core Team. 2008. R: A Language and Environment for Statistical Computing. R Development Core Team, Vienna, Austria.

Ricker, W. E. 1981. Changes in the average size and average age of pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences **38**:1636–1656.

Rijnsdorp, A. 1993. Fisheries as a large-scale experiment on life-history evolution: disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia **96**:391–401.

Rijnsdorp, A. D., and F. Storbeck. 1995. Determining the onset of sexual maturity from otoliths of individual female North Sea plaice, *Pleuronectes platessa* L. In D. H. Secor, J. M. Dean, and S. E. Campana, eds. Recent Developments in Fish Otolith Research, pp. 271–282. University of South Carolina Press, Columbia.

Rubin, J.-F., and N. Perrin. 1990. How does the body-scale model affect back-calculated growth: the example of arctic charr, *Salvelinus alpinus* (L.), of Lake Geneva (Switzerland). Aquatic Sciences **52**:287–295.

Smith, T. B., and L. Bernatchez. 2008. Evolutionary change in human-altered environments. Molecular Ecology **17**:1–8.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, New York.

Stockwell, C. A., A. P. Hendry, and M. T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology & Evolution **18**:94–101.

Stokes, K., and R. Law. 2000. Fishing as an evolutionary force. Marine Ecology Progress Series **208**:307–309.

Swain, D. P., A. F. Sinclair, and J. M. Hanson. 2007. Evolutionary response to size-selective mortality in an exploited fish population. Proceedings of the Royal Society B: Biological Sciences **274**:1015–1022.

Swain, D. P., A. F. Sinclair, and J. M. Hanson. 2008. Evolution of growth in Gulf of St Lawrence cod: reply to Heino et al. Proceedings of the Royal Society B: Biological Sciences **275**:1113–1115.

Theriault, V., D. Garant, L. Bernatchez, and J. J. Dodson. 2007. Heritability of life-history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). Journal of Evolutionary Biology **20**:2266–2277.

Thomas, G., and R. Eckmann. 2007. The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth – the Lake Constance example revisited. Canadian Journal of Fisheries and Aquatic Sciences **64**:402–410.

Thompson, J. N. 1998. Rapid evolution as an ecological process. Trends in Ecology & Evolution **13**:329–332.

Thorpe, J. E. 1998. Salmonid life-history evolution as a constraint on marine stock enhancement. Bulletin of Marine Science **62**:465–475.

Thresher, R. E., J. A. Koslow, A. K. Morison, and D. C. Smith. 2007. Depth-mediated reversal of the effects of climate change on long-term growth rates of exploited marine fish. Proceedings of the National Academy of Sciences of the United States of America **104**:7461–7465.

Trippel, E. A. 1995. Age at maturity as a stress indicator in fisheries. BioScience **45**:759–771.

Walsh, M. R., S. B. Munch, S. Chiba, and D. O. Conover. 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecology Letters **9**:142–148.

Yoneda, M., and P. J. Wright. 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Marine Ecology Progress Series **276**:237–248.

Chapter 2

Change in individual growth rate and its link to gill-net fishing in two sympatric whitefish species

Sébastien Nusslé, Amanda Brechon, Claus Wedekind

Published in Evolutionary Ecology

Authors' contribution

SN and AB gathered the data.
SN analysed the data.
SN, AB and CW discussed the study and the manuscript.
SN, AB and CW wrote the manuscript.

Evol Ecol
DOI 10.1007/s10682-010-9412-3

ORIGINAL PAPER

Change in individual growth rate and its link to gill-net fishing in two sympatric whitefish species

Sébastien Nusslé · Amanda Bréchon · Claus Wedekind

Received: 7 March 2010 / Accepted: 14 July 2010

Abstract Size-selective fishing is expected to affect traits such as individual growth rate, but the relationship between the fishery-linked selection differentials and the corresponding phenotypic changes is not well understood. We analysed a 25-year monitoring survey of sympatric populations of the two Alpine whitefish *Coregonus albellus* and *C. fatioi.* We determined the fishing-induced selection differentials on growth rates, the actual change of growth rates over time, and potential indicators of reproductive strategies that may change over time. We found marked declines in adult growth rate and significant selection differentials that may partly explain the observed declines. However, when comparing the two sympatric species, the selection differentials on adult growth were stronger in *C. albellus* while the decline in adult growth rate seemed more pronounced in *C. fatioi.* Moreover, the selection differential on juvenile growth was significant in *C. albellus* but not in *C. fatioi*, while a significant reduction in juvenile growth over the last 25 years was only found in *C. fatioi.* Our results suggest that size-selective fishing affects the genetics for individual growth in these whitefish, and that the link between selection differentials and phenotypic changes is influenced by species-specific factors.

Keywords Rapid evolution · Artificial selection · Salmonid · *Coregonus* · Selection differential · Lake Brienz (Switzerland)

Electronic supplementary material The online version of this article (doi:10.1007/s10682-010-9412-3) contains supplementary material, which is available to authorized users.

S. Nusslé (✉) · A. Bréchon · C. Wedekind
Department of Ecology and Evolution, Biophore, University of Lausanne,
1015 Lausanne, Switzerland
e-mail: Sebastien.Nussle@unil.ch

A. Bréchon
e-mail: Amanda.Brechon@unil.ch

C. Wedekind
e-mail: Claus.Wedekind@unil.ch

Published online: 23 July 2010

Introduction

Fishing-induced mortality can be very high and often exceeds natural mortality significantly (Rijnsdorp 1993; Mertz and Myers 1998; Jackson et al. 2001). Fishing is typically size-selective (Myers and Hoenig 1997; Fukuwaka and Morita 2008), and since significant heritabilities have been reported for traits that could be size-related in many fish [up to $h^2 = 0.5$, see (Theriault et al. 2007; Carlson and Seamons 2008)], size-selective fishing is expected to induce rapid evolutionary changes (Palumbi 2001; Smith and Bernatchez 2008; Darimont et al. 2009). Traits such as age or size at maturation (Heino et al. 2002; Grift et al. 2003; Sharpe and Hendry 2009), average reproductive effort (Yoneda and Wright 2004; Thomas et al. 2009), or individual growth rates (Handford et al. 1977; Ricker 1981; Swain et al. 2007; Thomas and Eckmann 2007; Nusslé et al. 2009) are likely to evolve in response to size-selective fishing. Such fishing has therefore been termed a 'large-scale experiment in life-history evolution' (Rijnsdorp 1993; Law 2000; Stokes and Law 2000), and studies on fishery-induced evolution have increased in numbers during the last decade (see Jørgensen et al. (2007) for a review of phenotypic change attributed to fishery-induced selection).

Despite this increased interest in recent years, it is still largely unclear how much of the frequently observed change in individual growth rate is due to harvesting and how much to other environmental factors that have changed over time. For example, many freshwater systems have seen a change in phosphorus concentration and hence of biomass production over the last few decades (Müller et al. 2007a, b). Such changes in phosphorus concentration could contribute to changes in growth rates of many fish (Gerdeaux et al. 2006; Müller et al. 2007b; Thomas and Eckmann 2007). Separating the effects of fishery- versus environmentally-induced changes on individual growth rates is usually difficult (Heino et al. 2008) because phenotypic plasticity is important in fish (Thorpe 1998; Crozier et al. 2008). Even if genetic changes can be documented over time, monitoring data alone cannot conclusively demonstrate the causal link between such genetic changes and particular changes in the environment (Hutchings and Fraser 2008). However, a critical step forward in estimating the importance of evolution for phenotypic changes is to measure the strength of selection, namely the selection differential (Law 2000, 2007; Smith and Bernatchez 2008).

Alpine whitefish (*Coregonus* sp., Salmonidae) may be valuable models for studying human impacts on evolution of fish (Müller et al. 2007b; Thomas and Eckmann 2007; Nusslé et al. 2009; Thomas et al. 2009). Populations of Alpine whitefish are comparatively well-defined because they are usually confined to individual lakes, and genetic analyses suggest that there is often little gene flow between populations (Douglas 1998; Vonlanthen 2009; Bittner et al. 2010). Fishery on Alpine whitefish has generally been regulated and monitored for several decades. A typical pattern is that the fishing pressure on Alpine whitefish has been fairly constant and high during the last decades, i.e. most fish were harvested in their first years of life and old individuals are now scarce (Müller et al. 2007b; Nusslé et al. 2009). We analysed a 25-year long monitoring program to determine the selection differentials and the phenotypic changes over several generations of two sympatric Alpine whitefish species. We found significant selection differentials and a significant growth decrease in both species. Differences between the species suggest that fishing-induced evolution is to some degree species-specific.

Methods

We studied whitefish of Lake Brienz, Switzerland (46.43°N, 7.58°E; surface area = 29.8 km^2, elevation = 564 m, maximum depth = 261 m). Lake Brienz is one of the few Alpine lakes that was largely unaffected by the general eutrophication prior and up to the 1970s. Its relatively low phosphorus concentrations have even decreased since then (Fig. 1a), so that the lake can now be called "ultra-oligotrophic" (Müller et al. 2007a). A monitoring program, collecting 25 whitefish from ordinary fishery catches every month, has been conducted by the Fishery Inspectorate of the Bern Canton since 1984. Each individual is sexed (by dissection) and total body length and body weight are measured to the nearest mm and g, respectively. Gillrakers are counted for species identification (Müller 2003) and scale samples are taken (above the lateral line between the adipose and dorsal fin) for age and growth determination. The gillnets used by fisheries were set to 35–40 mm mesh size (as measured when stretched) for bottom nets and 38 mm for floating nets prior to 1992. In response to the observed growth decrease and the declining yield (Fig. 1a), the minimal legal mesh size was reduced to 30 mm for bottom nets in 1993, and for floating nets in 1996. The fishing pressure is not determined in detail, but fishermen seem to largely adapt their effort to the availability of the fish (C. Küng, Fishery Inspectorate Bern, Personal Communication). The number of fishermen declined from four until 1998 to three until 2005. During the last 5 years, only two fishermen remained.

Two groups of whitefish were sampled, the slow-growing "small-type" whitefish Brienzlig (*Coregonus albellus* Fatio) and the fast-growing "large-type" whitefish which are mostly, if not exclusively, Albock (*C. fatioi* Kottelat). The taxonomy of Alpine whitefish is often unclear and controversial. This is also true for the different whitefish of Lake Brienz although they can clearly be grouped according to life history, morphology, and genetics (Kottelat and Freyhof 2007; Bittner 2009; Vonlanthen 2009). While Bittner (2009), for example, call the various whitefish of Lake Brienz "forms" of *C. lavaretus*, Kottelat and Freyhof (2007) distinguish them as species. We do not attempt to solve this ongoing discussion here but use Kottelat and Freyhof's (2007) guide to name the two most abundant whitefish groups of Lake Brienz (see also the discussion in Fraser and Bernatchez (2001) on defining conservation units). There is a third and probably a fourth other whitefish species in Lake Brienz (Douglas 1998; Kottelat and Freyhof 2007) that are both difficult to distinguish from *C. fatioi* on the basis of phenotypic characteristics only, but these other species are comparatively rare and numerically not important for fishery (Müller et al. 2007b). Moreover, because we confined our scale measurements (see below) and all further analyses to fish that were caught in December and January only (N_{total} = 1,106, $N_{Brienzlig}$ = 727, N_{Albock} = 379), i.e. around the spawning time of the winter-spawning *C. albellus* and *C. fatioi*, we assume that large-type whitefish that are not *C. fatioi* are not present or rare in our samples and will therefore not significantly affect our analyses. *C. albellus* is a pelagic species with relatively dense gillrakers, indicating fish that are more efficient when feeding on zooplankton and *C. fatioi* has less gillrakers indicating fish that are thought to be more efficient on benthic food (Link and Hoff 1998).

For each individual fish we determined the average scale radius and all annulus radii, i.e. the distances from the nucleus to the subsequent annuli, using an ocular micrometer and two different scales per fish. We back-calculated the length at previous ages according to the method of Finstad (2003). This method is based on a multiple regression including the size of the scale, the age and length of the fish. This takes into account that scale growth might not be linearly related to fish growth as slower growing fish may have proportionately larger scales than faster growing individuals, or that there might be an

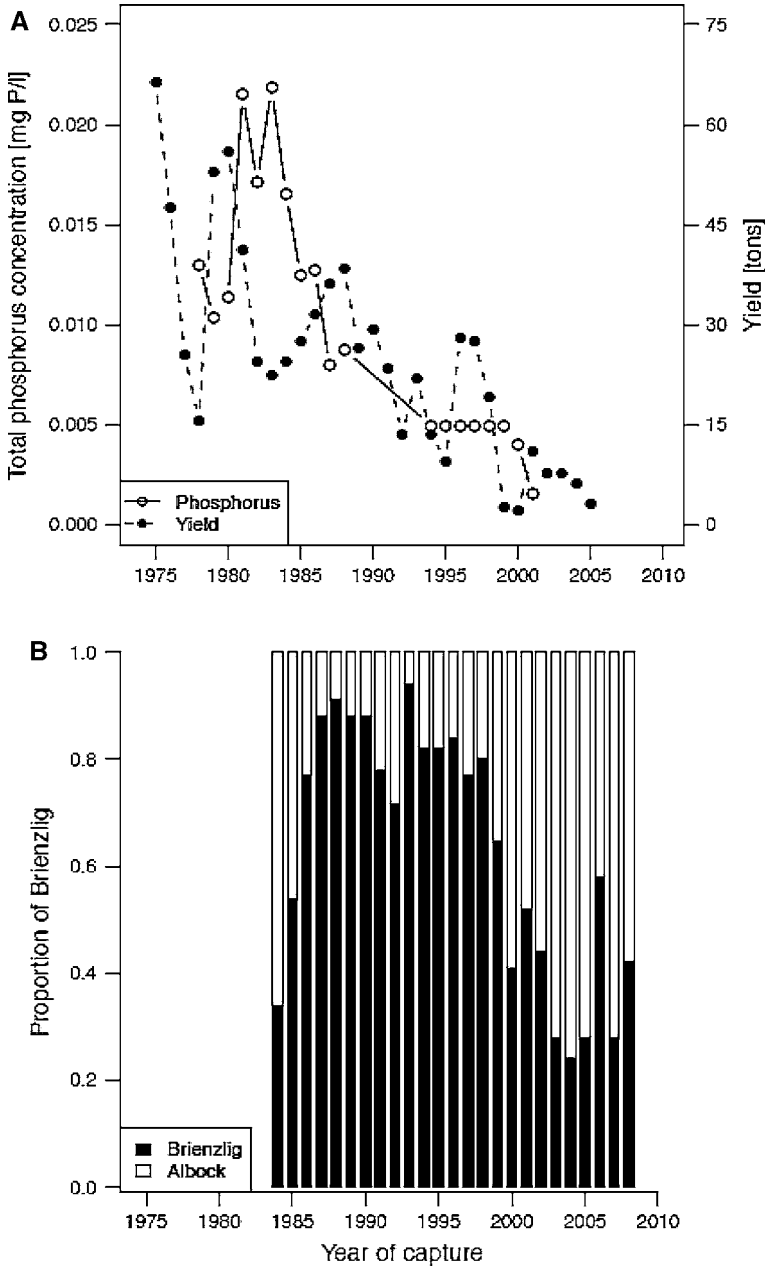


Fig. 1 Total phosphorus concentration, fishery yield, and relative abundances of the two whitefish species. **a** Total phosphorus (mg/l) (*empty circles*) in Lake Brienz during the last decades, redrawn from (Hoyle 2004) and fishing yield in Lake Brienz in tons (*full circles*). **b** Proportion of the total number of Brienzlig (*black*) and Albock (*white*) caught each year

age-specific growth of the scale irrespective of somatic growth. Then, to test for a potential bias in the back-calculations that could be linked to the age-at-capture, we analysed the residuals of the regression of the back-calculated lengths at capture (based on annuli radii) with the empirical length (based on the growth parameters) (Supplementary Fig. 1).

From the resulting length-at-age back-calculations, we computed the two-parameter logarithmic growth curve for each fish described in Nusslé et al. (2009):

$$L_i(t) = \alpha_{0i} + \alpha_{ti} \log(t) \quad (1)$$

where $L_i(t)$ is the back-calculated length of each fish at age t, α_{0i} is the back-calculated length at age 1, and α_{ti} is the logarithmic growth of the i-th fish. Parameter α_{ti} represents the length increase per time unit on a logarithmic scale. Hence α_0 represents juvenile growth while α_t is a measure of adult growth (Supplementary Fig. 2). The fit of this model was assessed with linear regressions of the back-calculated length (modelled) as a function of the length at capture (observed) (Supplementary Fig. 1). These back-calculated lengths fit well with the observed ones for both the Brienzlig (linear regression: slope $= 0.98 \pm 0.01$, $r^2 = 0.86$, Supplementary Fig. 1A) and the Albock (slope $= 0.93 \pm 0.02$, $r^2 = 0.83$, Supplementary Fig. 1B). The adult growth estimation based on all the annuli does not significantly differ from the estimations with a reduced number of annuli (Supplementary Fig. 3), suggesting that our estimation of the selection differentials is not biased in this regard. There is, however, a small but statistically significant effect of the age at capture on the residuals of the linear regression between back-calculated length and observed length for both the Brienzlig (linear regressions: $t_{347} = -3.6$, $p < 0.01$, $r^2 = 0.01$; Supplementary Fig. 1C) and the Albock ($t_{704} = -3.1$, $p < 0.001$, $r^2 = 0.03$; Supplementary Fig. 1D). The back-calculated lengths and the adult growth parameters are hence slightly underestimated for the oldest fish of our sample, but this barely affects our overall estimates of the selection differentials (see below) because the respective r^2s are rather small ($\leq$0.03) and older fish relatively scarce.

We used linear regressions of cohort-averaged growth parameters on year of birth of the fish to detect a potential change in growth parameters over time. In order to compare the two whitefish species, the observed growth changes were standardized to relative growth change (in %). We therefore divided, separately for Brienzlig and Albock, the observed change by the average growth parameters and multiplied this ratio with the respective average generation time. By assuming that average generation time remained constant over the observation period, an average generation time could be estimated separately for small-type and large-type whitefish according to Stearns (1992), assuming that only minor changes in survival and fecundity occurred during the monitoring:

$$\text{Generation time} = \frac{\sum_x x l_x m_x}{\sum_x l_x m_x} \quad (2)$$

where x is the age class of the fish, l_x is the probability of survival to age x, and m_x is the fecundity of age class x. Fecundity was estimated as the probability (p) of being mature at age x times the mean length (L) of the fish in the age class x cubed ($m_x = pL^3$), assuming that fecundity is proportional to the length^3 of the fish (Clark and Bernard 1992).

As a potential indicator for resource allocation from growth to reproduction, we estimated the reproductive investment of females as the condition factor Fulton K ($K = 10^5 \cdot$ fish weight/fish length^3). We also estimated the age at maturation for each fish according to the method of Rijnsdorp and Storbeck (1995) which assumes that growth is maximal and linear when the fish is immature and decreases after the fish becomes mature because some

resources are invested into reproduction instead of growth. We used linear regressions to test for linear trends over time in these two measures.

The selection differential s was determined for each age class within each cohort by comparing the reproducers, i.e. the fish caught in subsequent years and at older age, with all the fish of that particular age class. These estimates of s were then averaged for each cohort as in Nusslé et al. (2009). For each fish, we calculated individual growth parameters based on annuli radii and compared these growth parameters within cohorts. For each comparison between age classes, the estimation of the growth parameters was calculated with the same number of annuli in order to estimate the differences within cohort with the same metric. Analyses of variance of the estimated growth parameter as a function of the number of annuli indicated that effect of age had no effects our estimation of growth parameters (Supplementary Fig. 3). All analyses were run in the open-access statistical software "R" (R Development Core Team 2009). Population means are presented as mean ± standard deviation. All p-values are two-tailed.

Results

Overall, the samples consisted of 66% Brienzlig and 34% Albock (354 individuals). The relative prevalence of Brienzlig varied over the years and generally declined from the 1990s to the 2000s (Fig. 1b). The average generation time, i.e. the average age difference between parents and offspring was estimated to be 3.81 years for the slow-growing Brienzlig, and 3.59 years for the fast-growing Albock.

Length at age 1 (α_0) did not seem to change over the observational period for Brienzlig ($t_{23} = -0.5$, $p = 0.62$, Fig. 2a), while a slight but significant linear decrease of $-0.90 \pm 0.32\%$ per generation was observed for Albock ($t_{23} = -2.8$, $p = 0.01$, Fig. 2b). Large decreases in logarithmic growth (α_t) were observed for both species: $-7.71 \pm 1.7\%$ per generation for Brienzlig ($t_{23} = -4.4$, $p < 0.001$, Fig. 2c) and $-9.72 \pm 1.4\%$ per generation for Albock ($t_{23} = -7.1$, $p < 0.0001$, Fig. 2d).

Selection differentials on parameter α_0, i.e. the difference in juvenile growth between reproducers and the whole population, were small but significant for Brienzlig ($-3.9 \pm 1.1\%$, $t_{22} = -3.9$, $p = 0.001$, Fig. 3a) and not significantly different from zero for Albock ($t_{22} = -0.7$, $p = 0.49$, Fig. 3b). These α_0 were also not significantly different between the species ($t_{44} = 1.4$, $p = 0.16$). In contrast, selection differentials for logarithmic growth (α_t) were large and significantly negative for both Brienzlig ($-17.4 \pm 2.2\%$, $t_{22} = -17.4$, $p < 0.0001$, Fig. 3c) and Albock ($-7.0 \pm 2.4\%$, $t_{23} = -7.0$, $p = 0.008$, Fig. 3d). Moreover, the selection differentials were larger for Brienzlig than for Albock ($t_{45} = 2.4$, $p = 0.02$).

There seem to be slight changes for both the maturation schedule ($t_{23} = 1.9$, $p = 0.07$, Fig. 4a), and Fulton's condition factor ($t_{19} = -2.1$, $p = 0.04$, Fig. 4c) in Brienzlig. In this species, age at maturation increased by $0.23 \pm 0.12\%$ per year (but this apparent increase was not statistically significant), while the condition factor of the females during the winter months decreased by $0.61 \pm 0.29\%$ per year. No such change was found in Albock: neither the maturation schedule ($t_{23} = 0.9$, $p = 0.39$, Fig. 4b) nor Fulton's condition factor ($t_{20} = -1.5$, $p = 0.38$, Fig. 4d) changed significantly over time.

Discussion

The 25-year long monitoring program revealed significant negative selection differentials for individual growth in the two most common whitefish species of Lake Brienz. The

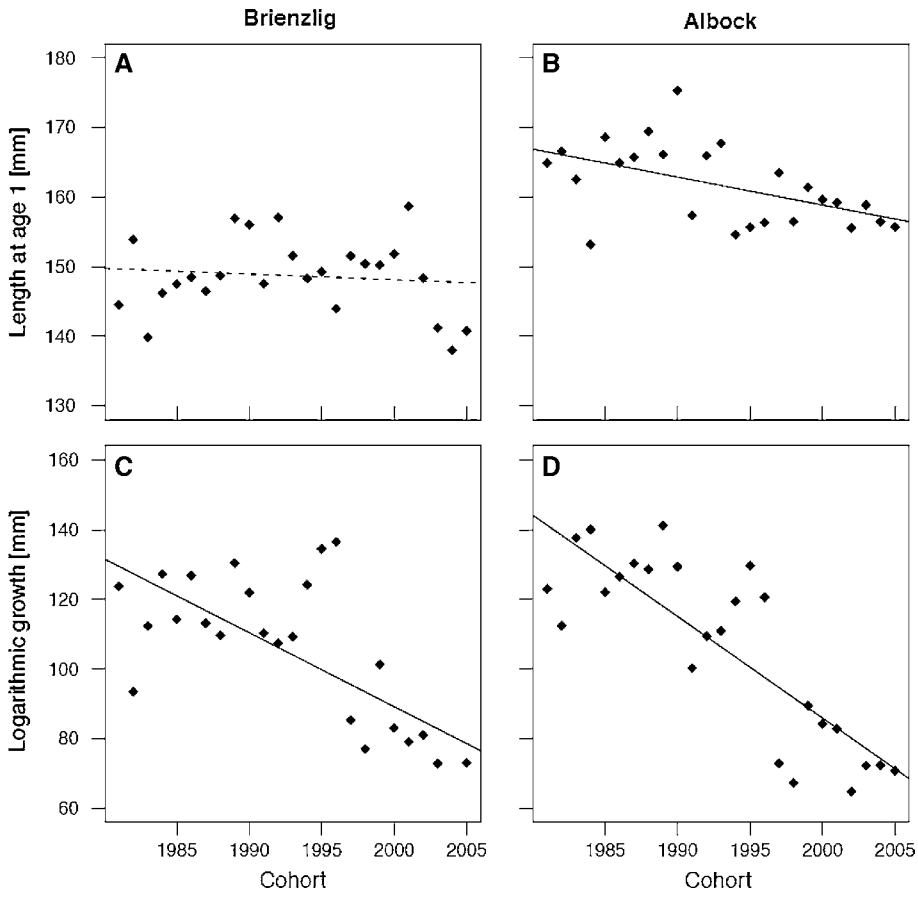


Fig. 2 Growth parameters over time. Growth parameters per cohort: **a**, **b** average length at age 1 (α_0) and **c**, **d** average logarithmic growth (α_t). The lines represent regression lines (solid = significant linear relationship, dashed = non-significant)

selection differential for adult growth in the fast-growing Albock seems only slightly higher than the selection differential of $-4.9 \pm 1.2\%$ that was found in a previous study on the Palée (*C. palaea* Fatio), a fast-growing whitefish in Lake Joux, Switzerland (Nusslé et al. 2009). The selection differential for adult growth that was found in the slow-growing Brienzlig is, however, around 3 times larger than in Albock and Palée. This difference in the strength of selection could be due to different phenotypic responses to environmental changes, differences in the ecological changes of the species-specific ecological niches, or qualitative differences in the fishing pressure, such as potential differences in the gillnet selectivity relative to age classes. The similar mean generation times that we found for Brienzlig and Albock (3.6 and 3.8 years, respectively) suggest, however, that the overall fishing pressure on these two species is about comparable. If mean generation time is indeed a useful indicator of the fishing pressure on whitefish, both Albock and Brienzlig seem to be under stronger fishing pressure than the Palée of Lake Joux for which Nusslé et al. (2009) found a mean generation time of 4.7 years.

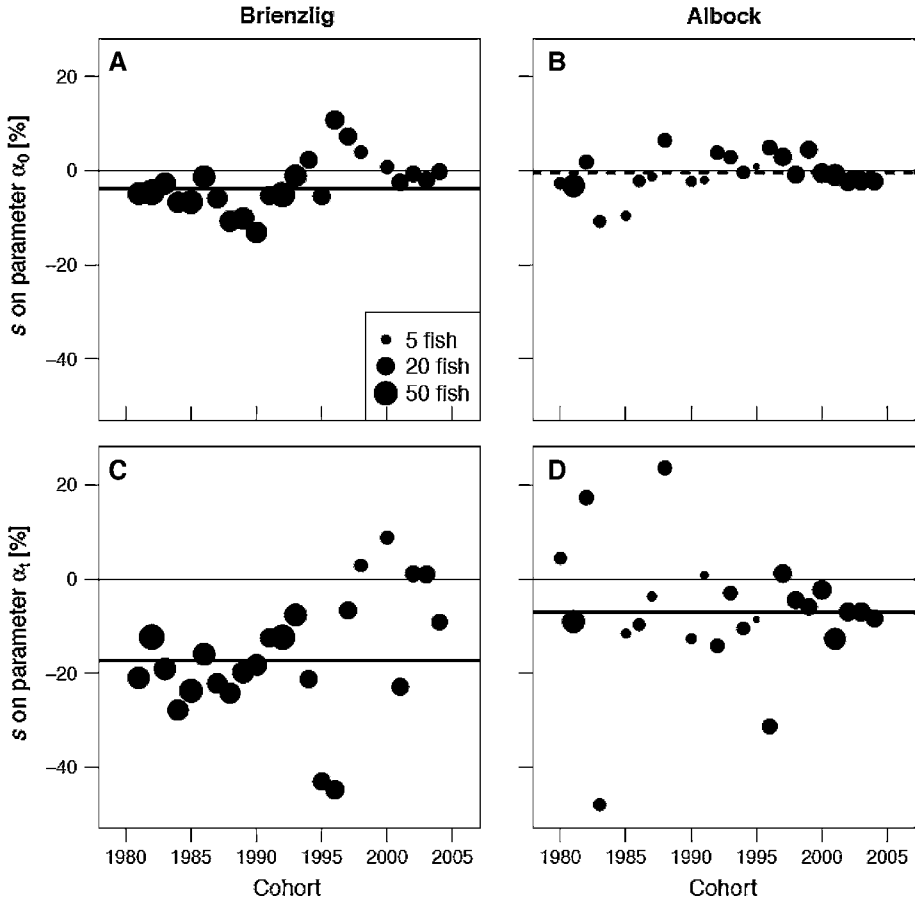


Fig. 3 Selection differentials (s) estimated for each cohort. **a**, **b** s for length at age 1 (α_0), and **c**, **d** s for logarithmic growth (α_t). The width of the circle corresponds to the number of fish within each cohort. The cohort is specified by the year of hatching from egg. The lines represent the average selection differential (solid = significantly different from zero, dashed = non-significant)

The selection differential on juvenile growth was not significantly different from zero in the Albock. This is in accordance with the previous findings on the Palée of Lake Joux (Nusslé et al. 2009). In the case of the slow-growing Brienzlig, the selection differential for juvenile growth was significantly different from zero but about 4 times smaller than the selection differential for adult growth. As juvenile growth is typically linked to juvenile survival (Tipping 2008), there might be compensatory mechanisms, or even selection against slow-growers, that could partly explain the pattern we observed here.

We found no or only a slight decline in juvenile growth rate but a marked decline in adult growth rate in both species. The decline in adult growth of both species of Lake Brienz was 2–2.5 times more pronounced than the decline found in the Palée of Lake Joux (Nusslé et al. 2009). This may again indicate differences in fishing intensity (see above) or could be linked to ecological differences between the lakes. For example, the total phosphorus concentration in Lake Joux halved from 30 to 35 mg/m^3 in the 1980s to

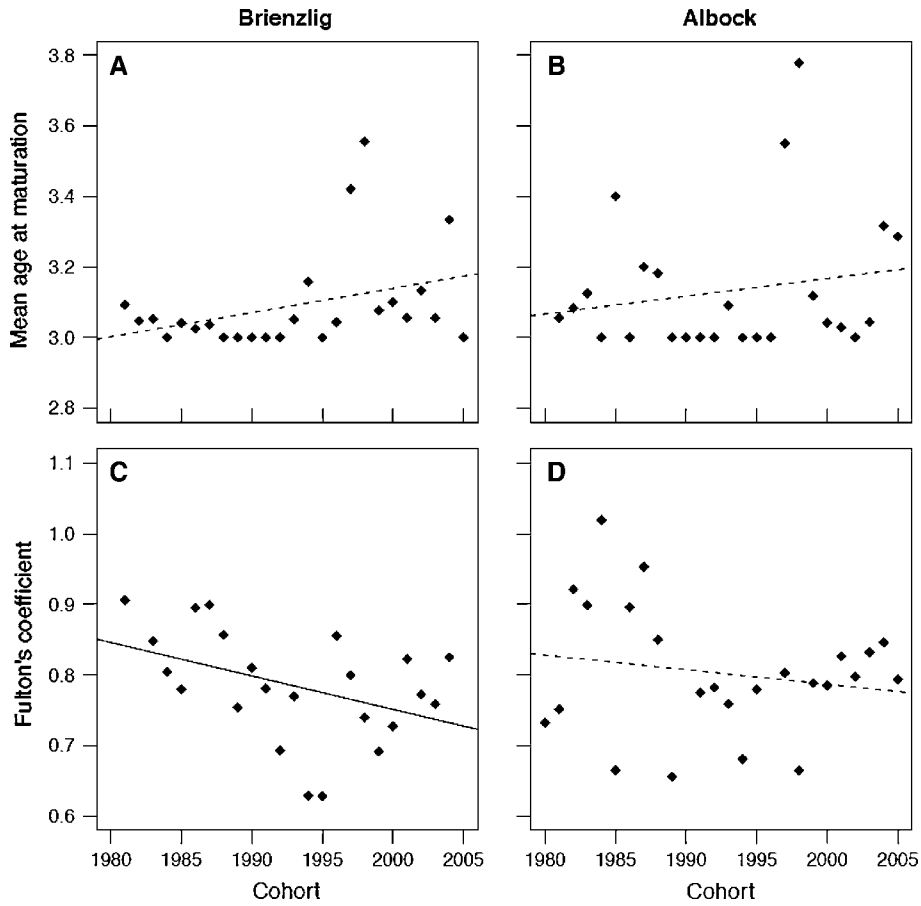


Fig. 4 Resource allocation from growth to reproduction. Indicators of resource allocation from growth to reproduction: **a** the mean age at maturation for each cohort and **b** the Fulton's coefficient of females during December and January. The lines represent regression lines (solid = significant linear relationship, dashed = non-significant)

15–20 mg/m^3 today (Lods-Crozet et al. 2006), while in Lake Brienz the corresponding decline was approximately by a factor of 4.

Fishery-induced selection and environmental change can both lead to a decrease in growth, and the relative contributions of genetic variation and phenotypic plasticity to changes in life history traits is mostly unclear (Hilborn 2006; Browman et al. 2008). However, assuming that genetic and environmental factors have additive effects on growth, and that interactions between genotype and environment can be ignored, a first estimate of the evolutionary response to fishery-induced selection R can be derived from the breeder's equation $R = h^2 s$ (Falconer and Mackay 1996), where h^2 is the narrow-sense heritability for growth rate. The fraction of change due to fishery-induced selection can then be estimated as $h^2 s$ divided by the total observed change in growth. As far as we know, no estimates exist for h^2 in Alpine whitefish. However, estimates of h^2 in other fish, including many other salmonids, range mostly from 0.1 to 0.5 and may be well-represented by $h^2 = 0.3$ (range: 0.1–0.5) (Law 2000; Garcia de Leaniz et al. 2007; Swain et al. 2007;

Theriault et al. 2007; Carlson and Seamons 2008). Our study populations provide measures of selection differentials because the large majority of fish are eventually harvested and old individuals are scarce (Nusslé et al. 2009). The response to selection on adult growth would then be a growth reduction of 5.2 ± 0.7% per generation for the Brienzlig (range: 1.7–8.7%), and 2.1 ± 0.7% for Albock (range: 0.7–3.5%). The proportion of the observed decline per generation in adult growth decrease that is due to fishery-induced selection would then be 67.7% (range 22.6–100%) for the Brienzlig and 21.6% (range: 7.2–36%) for the Albock. The corresponding value for the Palée of Lake Joux was 34% (range 11–56%) (Nusslé et al. 2009). All these estimates suggest that fishery-induced evolution plays a significant role in the contemporary evolution in Alpine whitefish.

We would like to stress that these estimates are based on various simplified model assumptions, apart from the fact that the h^2 of our study populations could differ from what is known about salmonids in general. We found a correlation between fishing yield, a potential measure of the density of fish, and the phosphorus concentration in the lake. Both factors are expected to influence fish growth, but in opposite directions: a reduced fish density might typically increase individual growth while a reduced phosphorus concentration might typically decrease it. It is unclear whether and by how much these opposing effects can cancel each other out so that the remaining variation in individual growth would then largely be linked to genetic factors only. In addition, different back-calculation methods could lead to slightly different estimates of the selection differentials. It is possible that our estimates of the selection differentials are somewhat biased if, for example, age-at-capture affects the estimation of the back-calculated lengths. Finally, we know little about possible genetic × environment interaction effects. All these potential drawbacks illustrate the difficulties encountered when estimating the contribution of genetic and environmental factors on growth in the changing environments of natural populations. However, our first estimates of the genetic effects of fishery-induced selection may at least indicate the range at which we expect that environmental and genetic factors interact. Moreover, the different responses that we observed in the two species suggests that fisheries impact might be species-specific.

The Brienzlig was on average more abundant than the Albock, but the relative contribution of Brienzlig to the total catch varied over the years and declined since the 1990s. This yearly variation could potentially be linked to the lake's total biomass production (i.e. to phosphorus concentrations) or to other ecological factors that changed over the observational period. Alpine whitefish are known to show high variation in the number of gillrakers, which are linked to food preference (Vonlanthen 2009; Bittner et al. 2010). A change in phosphorus concentration is likely to influence the primary production, which in turn may favour selection for one or the other species. The yearly variation in species abundance may also be influenced by the fishing regime on these two species. Indeed, the fishing pressure was not constant throughout the monitoring period as minimal mesh size was reduced in 1993 for bottom nets and in 1996 for floating nets. Our calculated selection differentials could therefore be underestimating the current strength of selection because a larger range of mesh size is currently allowed.

Fishery-induced selection and environmental change can lead to changes in reproductive strategies. We used mean age at maturation and the Fulton's K coefficient as first possible indicators of reproductive strategies (Gadgil and Bossert 1970; Heino et al. 2008). Females with many eggs are expected to be heavier and therefore to have a higher Fulton's coefficient. Our findings suggest that the individual reproductive investment of females did not increase over the years. In addition, the age at maturation did not significantly change over time. Overall, it seems that the resources allocated to growth have not been

re-invested into reproductive strategies over the observational period. The decrease observed in Fulton's coefficient of Brienzlig could be explained by fish being slimmer due to decreased nutrients concentration and/or adaptation to fishing gear (Hard et al. 2008).

A decrease in both adult and juvenile growth can have deleterious consequences for populations. There is now mounting evidence that artificial selection such as harvesting can reduce the average viability of populations (Fenberg and Roy 2008). Several specific consequences may arise from the removal of large fish, and even if these issues are still debated (Carlson et al. 2008), a precautionary approach should be taken when managing evolving fish stocks (Francis and Shotton 1997). First, large and fast-growing individuals may be of higher genetic quality than small and slow-growing individuals (Birkeland and Dayton 2005). A systematic removal of 'high quality' adults could therefore result in an increase of the average genetic load in a population. Second, as large females usually produce larger offspring of higher viability (Trippel 1995; Walsh et al. 2006), a decrease in growth could impair the recruitment and consequently the long-term yield of the population. Third, as females in many species prefer to mate with large males (Wedekind et al. 2007; Hutchings and Rowe 2008; Rudolfsen et al. 2008; Jacob et al. 2009; Labonne et al. 2009) increased mortality of large fish could have an impact on sexual selection and therefore on mating behaviour. Fourth, non-random mortality could decrease the genetic diversity of the population and make it more vulnerable to environmental changes or diseases (Jones et al. 2001).

Phenotypic plasticity can lead to reduced individual growth in lakes that experienced reduced biomass production due to reduced phosphorus input. However, our analyses suggest that phenotypic plasticity is not the only possible explanation for the significant reduction in individual growth rates that we observed. The large selection differentials that are imposed by size-selective fishing are likely to change the standing genetic variation for individual growth rates in the two whitefish species we studied here. Such fishery-induced evolution should be taken into account by population managers (Stokes and Law 2000; Ashley et al. 2003; Smith and Bernatchez 2008).

Acknowledgments We thank the Fishery Inspectorate of the Bern Canton for providing the monitoring data, E. Baumgartner for technical help, E. Clark, C. Küng, T. Mehner, R. Müller, M. Pompini, A. Ross-Gillespie, M. dos Santos, R. Stelkens, J. Van Buskirk, and two anonymous reviewers for discussion and/or comments on the manuscript, and the Swiss National Science Foundation for financial support.

References

Ashley MV, Wilson MF, Pergams ORW, O'Dowd DJ, Gende SM, Brown JS (2003) Evolutionarily enlightened management. Biol Conserv 111:115–123

Birkeland C, Dayton PK (2005) The importance in fishery management of leaving the big ones. Trends Ecol Evol 20:356–358

Bittner D (2009) Gonad deformations in whitefish (*Coregonus spp.*) from Lake Thun, Switzerland. A population genetic and transcriptomic approach. PhD thesis, University of Bern, Bern, Switzerland

Bittner D, Excoffier L, Largiader CR (2010) Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus* spp.) in a Swiss lake: a role for eutrophication? Mol Ecol 19:2152–2167

Browman HI, Law R, Marshall CT, Kuparinen A, Merila J, Jørgensen C, Enberg K, Dunlop ES, Arlinghaus R, Boukal DS, Brander K, Ernande B, Gårdmark A, Johnston F, Matsumura S, Pardoe H, Raab K, Silva A, Vainikka A, Dieckmann U, Heino M, Rijnsdorp AD (2008) The role of fisheries-induced evolution. Science 320:47–50

Carlson SM, Seamons TR (2008) A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evol Appl 1:222–238

Carlson SM, Olsen EM, Vøllestad LA (2008) Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. Funct Ecol 22:663–673

Clark JH, Bernard DR (1992) Fecundity of humpback whitefish and least cisco in the Chatanika River, Alaska. T Am Fish Soc 121:268–273
Crozier LG, Hendry AP, Lawson PW, Quinn TP, Mantua NJ, Battin J, Shaw RG, Huey RB (2008) Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evol Appl 1:252–270
Darimont CT, Carlson SM, Kinnison MT, Paquet PC, Reimchen TE, Wilmers CC (2009) Human predators outpace other agents of trait change in the wild. P Natl Acad Sci USA 106:952–954
Douglas M (1998) Central Alpine *Coregonus* (*Teleostei, Coregonidae*): evolution and conservation of a unique assemblage. PhD thesis, University of Zürich, Zürich, Switzerland
Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Benjamin Cummings, Essex
Fenberg PB, Roy K (2008) Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Mol Ecol 17:209–220
Finstad AG (2003) Growth backcalculations based on otoliths incorporating an age effect: adding an interaction term. J Fish Biol 62:1222–1225
Francis RICC, Shotton R (1997) ''Risk'' in fisheries management: a review. Can J Fish Aquat Sci 54:1699–1715
Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741–2752
Fukuwaka M, Morita K (2008) Increase in maturation size after the closure of a high seas gillnet fishery on hatchery-reared chum salmon *Oncorhynchus keta*. Evol Appl 1:376–387
Gadgil M, Bossert WH (1970) Life historical consequences of natural selection. Am Nat 104:1
Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher BH, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biol Rev 82:173–211
Gerdeaux D, Anneville O, Hefti D (2006) Fishery changes during re-oligotrophication in 11 peri-alpine Swiss and French lakes over the past 30 years. Acta Oecol 30:161–167
Grift RE, Rijnsdorp AD, Barot S, Heino M, Dieckmann U (2003) Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Mar Ecol Prog Ser 257:247–257
Handford P, Bell G, Reimchen T (1977) Gillnet fishery considered as an experiment in artificial selection. J Fish Res Board Can 34:954–961
Hard JJ, Gross MR, Heino M, Hilborn R, Kope RG, Law R, Reynolds JD (2008) Evolutionary consequences of fishing and their implications for salmon. Evol Appl 1:388–408
Heino M, Dieckmann U, Godo OR (2002) Measuring probabilistic reaction norms for age and size at maturation. Evolution 56:669–678
Heino M, Baulier L, Boukal DS, Dunlop ES, Eliassen S, Enberg K, Jørgensen C, Varpe O (2008) Evolution of growth in Gulf of St Lawrence cod? Proc R Soc Lond B Biol Sci 275:1111–1112
Hilborn R (2006) Faith-based fisheries. Fisheries 31:554–555
Hoyle C (2004) Phosphorus cycling in lake Brienz, Switzerland. MSc thesis, Swiss Federal Institute of Technology, Zurich, Kastanienbaum, Switzerland
Hutchings JA, Fraser DJ (2008) The nature of fisheries- and farming-induced evolution. Mol Ecol 17:294–313
Hutchings JA, Rowe S (2008) Consequences of sexual selection for fisheries-induced evolution: an exploratory analysis. Evol Appl 1:129–136
Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–638
Jacob A, Evanno G, Renai E, Sermier R, Wedekind C (2009) Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success in the minnow. Anim Behav 77:823–829
Jones MW, McParland TL, Hutchings JA, Danzmann RG (2001) Low genetic variability in lake populations of brook trout (*Salvelinus fontinalis*): a consequence of exploitation? Conserv Genet 2:245–256
Jørgensen C, Enberg K, Dunlop ES, Arlinghaus R, Boukal DS, Brander K, Ernande B, Gårdmark A, Johnston F, Matsumura S, Pardoe H, Raab K, Silva A, Vainikka A, Dieckmann U, Heino M, Rijnsdorp AD (2007) Ecology—managing evolving fish stocks. Science 318:1247–1248
Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. Kottelat and Freyhof, Cornol
Labonne J, Augery M, Parade M, Brinkert S, Prevost E, Heland M, Beall E (2009) Female preference for male body size in brown trout, *Salmo trutta*: is big still fashionable? Anim Behav 77:129–137
Law R (2000) Fishing, selection, and phenotypic evolution. Ices J Mar Sci 57:659–668

Law R (2007) Fisheries-induced evolution: present status and future directions. Mar Ecol Prog Ser 335:271–277
Link J, Hoff MH (1998) Relationships of Lake Herring (*Coregonus artedi*) gill raker characteristics to retention probabilities of zooplankton prey. J Freshwat Ecol 13:55–65
Lods-Crozet B, Reymond O, Strawczynski A (2006) Evaluation de la qualité chimique et biologique du lac de Joux (Jura Suisse) entre 1985 et 2004. Bull Soc Ne Sci Nat 129:29–47
Mertz G, Myers RA (1998) A simplified formulation for fish production. Can J Fish Aquat Sci 55:478–484
Müller R (2003) Populationsdynamische Untersuchungen an den Felchen des Brienzersees. In: Bericht an das Fischereiinspektorat des Kantons Bern. EAWAG, Kastanienbaum, Kastanienbaum, p 81
Müller B, Finger D, Sturm M, Prasuhn V, Haltmeier T, Bossard P, Hoyle C, Wuest A (2007a) Present and past bio-available phosphorus budget in the ultra-oligotrophic Lake Brienz. Aquat Sci 69:227–239
Müller R, Breitenstein M, Bia MM, Rellstab C, Kirchhofer A (2007b) Bottom-up control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquat Sci 69:271–288
Myers RA, Hoenig JM (1997) Direct estimates of gear selectivity from multiple tagging experiments. Can J Fish Aquat Sci 54:1–9
Nusslé S, Bornand C, Wedekind C (2009) Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. Evol Appl 2:200–208
Palumbi SR (2001) Evolution—humans as the world's greatest evolutionary force. Science 293:1786–1790
R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Ricker WE (1981) Changes in the average size and average age of pacific salmon. Can J Fish Aquat Sci 38:1636–1656
Rijnsdorp A (1993) Fisheries as a large-scale experiment on life-history evolution: disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia 96:391–401
Rijnsdorp AD, Storbeck F (1995) Determining the onset of sexual maturity from otoliths of individual female North Sea plaice, *Pleuronectes platessa* L. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. University of South Carolina Press, Columbia, S.C., pp 271–282
Rudolfsen G, Müller R, Urbach D, Wedekind C (2008) Predicting the mating system from phenotypic correlations between life-history and sperm quality traits in the Alpine whitefish *Coregonus zugensis*. Behav Ecol Sociobiol 62:561–567
Sharpe DMT, Hendry AP (2009) Life history change in commercially exploited fish stocks: an analysis of trends across studies. Evol Appl 2:260–275
Smith TB, Bernatchez L (2008) Evolutionary change in human-altered environments. Mol Ecol 17:1–8
Stearns SC (1992) The evolution of life histories. Oxford University Press, New York
Stokes K, Law R (2000) Fishing as an evolutionary force. Mar Ecol Prog Ser 208:307–309
Swain DP, Sinclair AF, Hanson JM (2007) Evolutionary response to size-selective mortality in an exploited fish population. Proc R Soc Lond B Biol Sci 274:1015–1022
Theriault V, Garant D, Bernatchez L, Dodson JJ (2007) Heritability of life-history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). J Evol Biol 20:2266–2277
Thomas G, Eckmann R (2007) The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth—the Lake Constance example revisited. Can J Fish Aquat Sci 64:402–410
Thomas G, Quoss H, Hartmann J, Eckmann R (2009) Human-induced changes in the reproductive traits of Lake Constance common whitefish (*Coregonus lavaretus*). J Evol Biol 22:88–96
Thorpe JE (1998) Salmonid life-history evolution as a constraint on marine stock enhancement. Bull Mar Sci 62:465–475
Tipping JM (2008) The effect of juvenile length in autumn on 2-year-old male and adult Coho salmon survival rates at three Washington hatcheries. N Am J Fish Aquacult 70:111–114
Trippel EA (1995) Age at maturity as a stress indicator in fisheries. Bioscience 45:759–771
Vonlanthen P (2009) On speciation and its reversal in adaptive radiations. The central European whitefish system. PhD Thesis, University of Bern, Bern, Switzerland
Walsh MR, Munch SB, Chiba S, Conover DO (2006) Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecol Lett 9:142–148
Wedekind C, Rudolfsen G, Jacob A, Urbach D, Müller R (2007) The genetic consequences of hatchery-induced sperm competition in a salmonid. Biol Conserv 137:180–188
Yoneda M, Wright PJ (2004) Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Mar Ecol Prog Ser 276:237–248

Addenda to chapter 2

When we tested for a potential bias in the back-calculations that could be linked to the age-at-capture, we analysed the residuals of the regression of the back-calculated lengths at capture (based on annuli radii) with the empirical length (based on the growth parameters). This figure can be found in the supplementary material online. However, a slight mistake was inserted in both the legend of the figure and the labels of the axes.

Back-calculated length at capture is correct and represent the estimated lengths based on annuli radii, however observed length at capture are wrongly designated and represent instead the empirical lengths, i.e. the theoretical lengths based on the two parameter growth model describe in this paper.

We apologise for the mistake and for the misunderstood that it may have caused.

Chapter 3

Fishery-induced evolution in Alpine whitefish: the role of environmental change and species-specific ecology

Sébastien Nusslé, Olivier Darbellay & Claus Wedekind

Manuscript

Authors' contribution

SN gathered the data, analysed the data and wrote the manuscript
OD provided technical help
CW supervised the project

Author's contributions have been thoroughly discussed before agreement.

Fishery-induced evolution in Alpine whitefish: the role of environmental change and species-specific ecology.

Running head: Fishery-induced evolution in whitefish

Sébastien Nusslé, Olivier Darbelay, Claus Wedekind

Department of Ecology and Evolution, University of Lausanne, Biophore, 1015 Lausanne, Switzerland

Corresponding author: Sébastien Nusslé, snussle@gmail.com

Original research paper

Acknowledgments

We thank the Fishery Inspectorate of the Bern Canton for providing the monitoring data, E. Baumgartner for technical help, E. Clark, V. Helfer, C. Küng, M. Pompini, A. Ross-Gillespie, M. dos Santos, R. Stelkens, T. Székely Jr. for discussion and/or comments on the manuscript, and the Société Académique Vaudoise for financial support.

Abstract

Size-selective fishing pressure is expected to affect the evolutionary trajectory of fish life-history traits but the exact relationship between fishery-linked selection differentials and corresponding phenotypic changes is not yet well understood. We analysed 25 years of monitoring data on sympatric populations of Alpine whitefish (*Coregonus palaea* and *C. confusus)* that specialize on different prey sizes. Using individual growth trajectories reconstructed from scale rings, we estimated fishing-induced selection differentials on growth rates, and compared these to observed changes in growth rates over time. We found significant negative selection differentials and marked declines in adult growth rate. Our results, taken alongside previous results from three other *Coregonus* species, confirm that size-selective fishing does indeed induce genetically-linked growth rate decreases in Alpine whitefish; but also show how its impact may be modulated by the environment, and by species-specific ecological traits such as trophic niche.

Keywords: rapid evolution, artificial selection, salmonid, *Coregonus*, selection differential, Lake Biel (Switzerland), phosphorus, eutrophic, oligotrophic

Introduction

Many fish populations are subject to fishery-induced size-selective mortality (Myers and Hoenig 1997; Fukuwaka and Morita 2008) that is often larger than natural mortality (Rijnsdorp 1993; Mertz and Myers 1998; Jackson *et al.* 2001). Significant heritabilities have been reported for life-history traits in many fish populations (Theriault *et al.* 2007; Carlson and Seamons 2008) indicating that selection on these traits is likely to induce change over generations. As many life-history traits are linked to fish size, size-selective fishing is known to induce rapid evolutionary changes on several life-history traits (Palumbi 2001; Smith and Bernatchez 2008; Darimont *et al.* 2009). These traits include age and size at maturation (Heino *et al.* 2002; Grift *et al.* 2003; Sharpe and Hendry 2009), average reproductive effort (Yoneda and Wright 2004; Thomas *et al.* 2009), spawning behaviour (Opdal 2010), and individual growth rates (Handford *et al.* 1977; Ricker 1981; Swain *et al.* 2007; Thomas and Eckmann 2007; Nusslé *et al.* 2009; Nusslé *et al.* in press). Professional fishing has therefore been called a 'large-scale experiment in life-history evolution' (Rijnsdorp 1993; Law 2000; Stokes and Law 2000) – a perspective that has received considerable attention during the last decade (Jørgensen *et al.* 2007).

Although fishery-induced evolution is well documented for many species (Jørgensen *et al.* 2007), there is still debate on its relative importance compared to the selective pressures of environmental change (Hilborn 2006; Browman *et al.* 2008; Smith and Bernatchez 2008; Andersen and Brander 2009). Disentangling the effects of fishery- versus environmentally-induced changes on individual growth rates is particularly difficult (Heino et al. 2008) because (a) phenotypic plasticity is frequently observed in fish (Thorpe 1998; Crozier et al. 2008) and (b) fishing-induced selective

pressure is often correlated with long-term environmental changes (Hutchings and Fraser 2008). A critical step forward in estimating the importance of evolution for phenotypic changes compared to environmental changes is to measure the strength of selection, i.e. the selection differential (Law 2000; Law 2007; Smith and Bernatchez 2008).

Swiss Alpine whitefish populations (*Coregonus* sp., Salmonidae) are excellent models to study the influence of both fishery-induced evolution and environmental changes (Müller *et al.* 2007b; Thomas and Eckmann 2007; Nusslé *et al.* 2009; Thomas *et al.* 2009; Nusslé *et al.* in press). First, these populations may provide relatively accurate measures of selection differentials as migration is negligible and the large majority of fish are harvested, rather than die of senescence (Müller *et al.* 2007b; Nusslé *et al.* 2009; Nusslé *et al.* in press). Second, harvesting of many Alpine whitefish populations has been relatively constant and well monitored for several decades. Third, the sample size of well-monitored populations is relatively large as many lakes have several sympatric populations with little gene flow between populations (Douglas *et al.* 1999; Douglas *et al.* 2005; Hudson *et al.* 2007; Vonlanthen *et al.* 2009; Bittner *et al.* 2010). Finally, these populations have experienced substantial environmental change over recent decades. In particular, phosphorus concentrations in several Swiss lakes have dramatically changed between the 1980s and the present day, and this environmental parameter is known to have a major impact on whitefish populations (Gerdeaux *et al.* 2006; Müller *et al.* 2007a; Müller *et al.* 2007b; Thomas and Eckmann 2007). Phosphorus is directly linked to biomass production of phytoplankton, which in turn is known to differentially affect different fish families trough trophic chain (Gerdeaux *et al.* 2006). For instance, phosphorus concentrations between 20-40 mg/m^3 are reportedly optimal for

coregonids (Gerdeaux *et al.* 2006; Eckmann and Rösch 2007). Above this limit, in eutrophic lakes for instance, competition with other fish (e.g. percids), and low recruitment linked to poor water quality combine to reduce coregonid density and therefore yield (Gerdeaux *et al.* 2006). On the other hand, in oligotrophic lakes, where phosphorus concentration is lower than 20 mg/m^3, primary productivity of the ecosystem is not sufficient to sustain large coregonid populations (Müller *et al.* 2007b). The low productivity in such lakes also impacts on the growth rate of fish, since less food means less growth. More subtly, changing phosphorous concentrations can have different effects on coregonids occupying different ecological niches. When food limited, zooplanktivorous fish preferentially eliminate larger prey, such that average prey size decreases (Jeppesen *et al.* 2000; Jeppesen *et al.* 2002; Müller *et al.* 2007b), potentially favouring fish that specialise in smaller prey.

In this study, we analysed data from a 25-year long monitoring survey to determine the selection differentials and the phenotypic changes, over several generations, of two sympatric Alpine whitefish species. The two species specialized on different prey sizes (Vonlanthen *et al.* 2009), where the balance between prey types was modulated by phosphorus concentration. We found significant selection differentials and significant growth decreases in both species. Differences in the response to fishing-induced evolution between populations facing different environmental and ecological conditions suggest that interactions between these variables may play an important role in determining evolutionary trajectory.

Methods

We compared data from whitefish surveys in three different lakes in Switzerland: Lake Joux (Nusslé *et al.* 2009) and Lake Brienz (Nusslé *et al.* 2010)

were analysed in previous studies, but this study focuses on Lake Biel. Lake Biel (46.43°N, 7.58°E; surface area = 37.8 km^2, elevation = 429 m, maximum depth = 74 m) is a mesotrophic lake that went through a period of relatively severe eutrophication during the 1970s-1980s (Figure 1) (Roth and Geiger 1972; Wright *et al.* 1981; Gerdeaux *et al.* 2006). Two sympatric species of whitefish are present in this lake, the slowly growing pelagic whitefish Bondelle (*Coregonus confusus* Fatio) and the fast growing benthic whitefish Palée (*C. palaea* Fatio). These species diverged after the last glaciations, around 15000 years ago (Pigeon *et al.* 1997), and since then have become adapted to different points of an ecological gradient, most likely in response to competition for food (Landry et al. 2007; Vonlanthen et al. 2009). While *C. confusus* display relatively dense gillraker arrays, indicating a trophic specialisation towards smaller zooplankton prey (mostly Cladocera), *C. palaea* has fewer gillrakers and is thought to be adapted to prey on larger benthic animals (e.g. chironomid larvae, copepods and molluscs) (Link and Hoff 1998; Tolonen 1998; Bernatchez *et al.* 1999; Eckmann *et al.* 2002; Müller *et al.* 2007b), although *C. palaea* may still turn to smaller prey if larger prey are lacking (Mookerji et al. 1998).

Since 1984, the Fishery Inspectorate of the Bern Canton (Switzerland) has collected a random sample of 25 whitefish from each months' regular catch. Each sampled individual is sexed (by dissection) and total body length and body weight are measured. Gillrakers are counted for species identification (Kottelat and Freyhof 2007) and scale samples are taken for age and growth rate determination, from above the lateral line between adipose and dorsal fins. For fish caught in winter (December and January), we determined the average scale radius and all annulus radii, i.e. the distances from the nucleus to the each concentric growth ring. We read two different scales per fish using an ocular micrometer and back-calculated body length at

previous ages according to the method of Finstad (2003). From the resulting length-at-age back-calculations, we estimated juvenile and adult growth rates according to the two-parameter growth model of Nusslé et al. (2009):

$$L_i(t) = \alpha_{0i} + \alpha_{ti} \log(t) \quad (1)$$

where $L_i(t)$ is the back-calculated length of each fish at age t, α_{0i} represents juvenile growth, and α_{ti} is a measure of adult growth. Parameter α_{0i} is the back-calculated length at age 1 and α_{ti} represents the length increase per time unit on a logarithmic scale.

We calculated the average growth parameter per cohort (i.e. the year of birth of the fish) and used linear regressions of growth parameters on the year of birth to detect potential changes in growth parameters over time. In order to compare the two whitefish species, the observed growth changes were standardized to relative growth change (in % per generation). We therefore divided, separately for Bondelle and Palée, the observed change by the average growth parameters and multiplied this ratio with the respective average generation time. By assuming that average generation time remained constant over the observation period and that only minor changes in survival and fecundity occurred during the monitoring period, an average generation time could be estimated separately for the two sympatric species according to Stearns (1992):

$$\text{Generation time} = \frac{\sum_x x l_x m_x}{\sum_x l_x m_x} \quad (2)$$

where x is the age class of the fish, l_x is the probability of survival to age x, and m_x is the fecundity of age class x. Fecundity was estimated as the probability (p) of being mature at age x times the mean length (L) of the fish in the age class x cubed ($m_x = pL^3$), assuming that fecundity is proportional to the length^3 of the fish (Clark and

Bernard 1992). As fishing targets preferentially larger, older fish, high fishing pressures should result in short generation times (Nusslé *et al.* 2010). Generation time was then also used as a first indicator of fishing intensity.

Fishing pressure can also potentially lead to changes in reproductive strategies: under increased mortality, females are expected to invest more in reproduction than maintenance (Heino *et al.* 2008). In other words, an observed decrease in growth rate may not solely reflect selection against fast growers, but could also reflect a change in females' allocation strategy towards more or earlier reproduction (Gadgil and Bossert 1970; Heino *et al.* 2008). In whitefish, however, recent studies have shown a relatively constant reproductive investment which did not interact with fishery- or environmentally-induced growth changes (Nusslé *et al.* 2009; Nusslé *et al.* in press). Nonetheless, in order to control for potential re-allocation from growth to reproduction, we estimated the reproductive investment of females using the condition factor Fulton's K ($K = 10^5 \cdot$ fish weight / fish length^3). Females with many eggs are expected to be heavier and therefore to have a higher Fulton's K. We also estimated the age at maturation for each fish according to the method of Rijnsdorp and Storbeck (1995). This method assumes that growth is maximal and linear when the fish is immature and decreases after the fish becomes mature because some resources are invested into reproduction instead of growth. The age at maturation is thus the age at which growth rate starts to decline from its maximum. We used linear regressions to test for linear trends over time in these two measures.

The selection differential s was determined for each age class within each cohort by comparing the reproducers (i.e. fish caught in subsequent years and at older age) with all the fish of that particular age class. These estimates of s were then averaged for each cohort as in Nusslé et al. (2009). For each fish, we calculated

individual growth parameters based on annuli radii and compared these growth parameters within cohorts. For each comparison between age classes, the estimation of the growth parameters was calculated with the same number of annuli in order to estimate the differences within cohort with the same metric.

With our selection differential *s* we went on to estimate the expected evolutionary response to fishery-induced selection *R* from the breeder's equation $R = h^2 s$ (Falconer and Mackay 1996), where h^2 is the narrow-sense heritability for growth rate. To our knowledge, no estimates exist for the heritability of Alpine whitefish growth, so we assumed that this value was comparable to the observed heritabilities for growth in several other salmonids, i.e. a value of $h^2 = 0.3$, with a range between 0.1 and 0.5 (Law 2000; Garcia de Leaniz *et al.* 2007; Swain *et al.* 2007; Theriault *et al.* 2007; Carlson and Seamons 2008). Assuming that genetic and environmental factors have additive effects on growth and that interactions between individual genotype and environment can be ignored, the fraction of change due to fishery-induced selection can then be estimated as $h^2 s$ divided by the total observed change in growth (see Nusslé et al. (2009; in press) for details).

All analyses were run in the open-access statistical software "R" (R Development Core Team 2010). Population means are presented as mean ± standard deviation. All p-values are two-tailed.

Results

Juvenile growth estimates (α_0) show relatively small but significant linear decreases over the observational period for both species: -1.85 ± 0.70% per generation for Bondelle ($t_{24} = -2.6$, $P < 0.05$, Fig. 2A) and -3.27 ± 1.00% per generation for Palée ($t_{24} = -3.3$, $P < 0.01$, Fig. 2B). Large decreases in adult growth (α_t) were observed for

both species: -5.41 ± 0.53% per generation for Bondelle (t_{24} = -10.3, P < 0.001, Fig. 2C) and -7.32 ± 1.11% per generation for Palée (t_{24} = -6.6, P < 0.0001, Fig. 2D). The average generation time, i.e. the average age difference between parents and offspring was estimated to be 3.79 years for the slow-growing Bondelle, and 3.77 years for the fast-growing Palée.

As in previous studies on Alpine whitefish (Nusslé *et al.* 2009; Nusslé *et al.* 2010), we again found no changes in the maturation schedule for either the Bondelle (t_{23} = -0.04, P = 0.97) or the Palée (t_{23} = -0.45, P = 0.66). Fulton's condition factor did not change for the Palée (t_{25} = -0.38, P = 0.71), but slightly increased of 0.4% per year for the Bondelle (t_{26} = 2.1, P = 0.04).

Selection differentials on parameter α_0, i.e. the difference in juvenile growth between reproducers and the whole population, were small but significant for Bondelle (-1.13 ± 0.38%, t_{24} = -3.0, P < 0.01, Fig. 3A) and almost significant for Palée (1.06 ± 0.53, t_{23} = -2, P = 0.057, Fig. 3B). In contrast, selection differentials for adult growth (α_t) were large and significantly negative for both Bondelle (-4.93 ± 0.65%, t_{24} = -7.6, P < 0.001, Fig. 3C) and Palée (-4.81 ± 0.91%, t_{23} = -5.3, P < 0.001, Fig. 3D). Table 1 contrasts the results from this study with those from previous studies.

Discussion

Twenty-five years of monitoring revealed that not only fishing exerts a noteworthy selective pressure on growth in our whitefish populations, but also that this pressure could be influenced by species specific and environmental factors. We found significant negative selection differentials for adult growth in the two sympatric species of Lake Biel, Bondelle and Palée, and also for juvenile growth in Bondelle.

For Palée selection differential on juvenile growth was almost significant. For adult growth the selection differentials, 4.9% for Bondelle and 4.8% for Palée, are similar to the selection differentials, 4.9%, observed for the Palée (*C. palaea*) of lake Joux (Nusslé *et al.* 2009) and slightly smaller than the selection differentials observed in lake Brienz for *C. albula* (17.4%) and *C. fatioi* (7.0%) (Nusslé *et al.* in press). The observed selection differentials for juvenile growth were much smaller in Lake Biel with value of 1.1% for both species. In the other lakes selection differentials were also much smaller and even not significantly different from zero. As expected if growth is heritable, we observed significant growth decreases for both species and for both adult and juvenile growth. The proportion of the observed decline per generation that is due to fishery-induced selection can be estimated and is equal for adult to 27.3% (range 9% - 46%) for the Bondelle and 19.7% (range 7% - 33%) for the Palée. The corresponding value for the Palée of Lake Joux was 34% (Nusslé et al. 2009), for the Brienzlig: 68% and for the Albock of Lake Brienz: 22% (Nusslé *et al.* in press). For the decrease observed in juvenile growth, these proportions would be 18.3% for the Bondelle and 9.8% for the Palée. In the two other lakes these values for juvenile growth could not be estimated as either selection differentials or growth decreases were not significantly different from zero. All these estimates suggest that fishery-induced evolution plays an important role in the contemporary evolution of growth in Alpine whitefish and should be taken into account by population managers (Stokes and Law 2000; Ashley *et al.* 2003; Smith and Bernatchez 2008).

Our results also suggest that the different patterns of selection strengths observed between coregonid species might be linked to their specialization for different preys sizes, which in turn is linked to phosphorus concentration. In lake Biel, selection differentials are similar for both species and the mean generation time seems

to be relatively constant within lakes indicating a relatively equivalent fishing pressure between the species present in the same lake. On the other hand, the slow-growing species (Bondelle and Brienzlig) display relatively smaller growth decreases compared to the one observed for the fast-growing species (Palée and Albock) (Table 1). If selection pressure is equivalent for the fast and the slow-growing species, a difference in the observed growth change indicates that slow-growing species are less influenced by phosphorus changes. Competition for food, a well-studied evolutionary force in Alpine whitefish (Landry *et al.* 2007; Vonlanthen *et al.* 2009) might explain these differences: phosphorus decreases primary productivity, and intensifies predation pressure on the largest individuals among the dwindling zooplankton stocks. In turn, selection shifts to favour smaller zooplankton (Jeppesen *et al.* 2000; Jeppesen *et al.* 2002; Müller *et al.* 2007b). Since the slow-growing pelagic species are specialized on smaller preys (Link and Hoff 1998; Tolonen 1998; Bernatchez *et al.* 1999; Eckmann *et al.* 2002; Müller *et al.* 2007b), these slow-growing species are likely to be less adversely affected by phosphorus decreases than the fast-growing ones that feed on larger prey (Mookerji *et al.* 1998).

Between-lake comparisons also suggest that the strength of selection and the resulting impact on growth might be influenced by environmental factors. In lake Brienz, fishing yield has dropped with phosphorus concentration (Figure 1), with relatively poor yields among the whitefish populations coinciding with periods when phosphorus concentration drops below 20 mg/m^3 (Gerdeaux *et al.* 2006; Eckmann and Rösch 2007). These poor conditions are associated with larger growth decreases and large selection differentials. In contrast, yield in lake Biel has increased despite constant fishing pressure (Figure 1), which may be due to improvements in phosphorous conditions over the years. Excessively high phosphorus concentrations

(> 100 mg/m^3)over past decades (figure 1) have indeed declined to within the optimal range in recent years (between 20-40 mg/m^3) (Gerdeaux *et al.* 2006; Eckmann and Rösch 2007). Similarly good conditions are found in lake Joux (Figure 1) and the selection differentials seems to be equivalent in these two lakes. In conclusion, fishing-induced selection seems to be linked to the environment, where harsher conditions seem to be linked to larger selection differentials.

Our results suggest that fishery-induced evolution can strongly select for reduced individual growth. Moreover, the process through which fishery-induced evolution and environmental changes interact to influence growth is likely to be complex and seems to be linked to the phosphorus concentration through competition for food and environmental pressure. The large selection differentials that are imposed by size-selective fishing are likely to drive heritable growth decreases. Population managers should take this into account for management plans; moreover, these management plans should be species-specific (Stokes and Law 2000; Ashley *et al.* 2003; Smith and Bernatchez 2008).

Bibliography

Andersen, K. H., and K. Brander. 2009. Expected rate of fisheries-induced evolution is slow. Proceedings of the National Academy of Sciences of the United States of America **106**:11657-11660.

Ashley, M. V., M. F. Wilson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation **111**:115-123.

Bernatchez, L., A. Chouinard, and G. Q. Lu. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, Coregonus sp., as a case

study. Biological Journal of the Linnean Society **68**:173-194.

Bittner, D., L. Excoffier, and C. R. Largiader. 2010. Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus spp.*) in a Swiss lake: a role for eutrophication? Molecular Ecology **19**:2152-2167.

Browman, H. I., R. Law, C. T. Marshall, A. Kuparinen, J. Merila, C. Jørgensen, K. Enberg, *et al.* 2008. The role of fisheries-induced evolution. Science **320**:47-50.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications **1**:222-238.

Clark, J. H., and D. R. Bernard. 1992. Fecundity of humpback whitefish and least cisco in the Chatanika River, Alaska. Transactions of the American Fisheries Society **121**:268-273.

Crozier, L. G., A. P. Hendry, P. W. Lawson, T. P. Quinn, N. J. Mantua, J. Battin, R. G. Shaw, *et al.* 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications **1**:252-270.

Darimont, C. T., S. M. Carlson, M. T. Kinnison, P. C. Paquet, T. E. Reimchen, and C. C. Wilmers. 2009. Human predators outpace other agents of trait change in the wild. Proceedings of the National Academy of Sciences of the United States of America **106**:952-954.

Douglas, M. R., P. C. Brunner, and L. Bernatchez. 1999. Do assemblages of Coregonus (Teleostei : Salmoniformes) in the Central Alpine region of Europe represent species flocks? Molecular Ecology **8**:589-603.

Douglas, M. R., P. C. Brunner, and M. E. Douglas. 2005. Evolutionary homoplasy among species flocks of central alpine Coregonus (Teleostei: Salmoniformes). Copeia:347-358.

Eckmann, R., M. Becker, and M. Schmid. 2002. Estimating food consumption by a heavily fished stock of zooplanktivorous Coregonus lavaretus. Transactions of the American Fisheries Society **131**:946-955.

Eckmann, R., and R. Rösch. 2007. Lake Constance fisheries and fish ecology.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Edited by Longman. 4th edition ed. Essex, UK: Benjamin Cummings.

Finstad, A. G. 2003. Growth backcalculations based on otoliths incorporating an age effect: adding an interaction term. Journal of Fish Biology **62**:1222-1225.

Fukuwaka, M., and K. Morita. 2008. Increase in maturation size after the closure of a high seas gillnet fishery on hatchery-reared chum salmon *Oncorhynchus keta*. Evolutionary Applications **1**:376-387.

Gadgil, M., and W. H. Bossert. 1970. Life historical consequences of natural selection. The American Naturalist **104**:1.

Garcia de Leaniz, C., I. A. Fleming, S. Einum, E. Verspoor, W. C. Jordan, S. Consuegra, N. Aubin-Horth, *et al.* 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews **82**:173-211.

Gerdeaux, D., O. Anneville, and D. Hefti. 2006. Fishery changes during re-oligotrophication in 11 peri-alpine Swiss and French lakes over the past 30 years. Acta Oecologica **30**:161-167.

Grift, R. E., A. D. Rijnsdorp, S. Barot, M. Heino, and U. Dieckmann. 2003. Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Marine

Ecology-Progress Series **257**:247-257.

Handford, P., G. Bell, and T. Reimchen. 1977. Gillnet fishery considered as an experiment in artificial selection. Journal of the Fisheries Research Board of Canada **34**:954-961.

Heino, M., L. Baulier, D. S. Boukal, E. S. Dunlop, S. Eliassen, K. Enberg, C. Jørgensen, *et al.* 2008. Evolution of growth in Gulf of St Lawrence cod? . Proceedings of the Royal Society of London B Biological Sciences **275**:1111-1112.

Heino, M., U. Dieckmann, and O. R. Godo. 2002. Measuring probabilistic reaction norms for age and size at maturation. Evolution **56**:669-678.

Hilborn, R. 2006. Faith-based fisheries. Fisheries **31**:554-555.

Hudson, A. G., P. Vonlanthen, R. Mueller, and O. Seehausen. 2007. Review: The geography of speciation and adaptive radiation in coregonines. Biology and Management of Coregonid Fishes - 2005 **60**:111-146.

Hutchings, J. A., and D. J. Fraser. 2008. The nature of fisheries- and farming-induced evolution. Molecular Ecology **17**:294-313.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, *et al.* 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629-638.

Jeppesen, E., J. P. Jensen, S. Amsinck, F. Landkildehus, T. Lauridsen, and S. F. Mitchell. 2002. Reconstructing the historical changes in Daphnia mean size and planktivorous fish abundance in lakes from the size of Daphnia ephippia in the sediment. Journal of Paleolimnology **27**:133-143.

Jeppesen, E., J. P. Jensen, M. Sondergaard, T. Lauridsen, and F. Landkildehus. 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes

along a phosphorus gradient. Freshwater Biology **45**:201-218.

Jørgensen, C., K. Enberg, E. S. Dunlop, R. Arlinghaus, D. S. Boukal, K. Brander, B. Ernande, *et al.* 2007. Ecology - Managing evolving fish stocks. Science **318**:1247-1248.

Kottelat, M., and J. Freyhof. 2007. Handbook of European freshwater fishes. Cornol, Switzerland: Kottelat and Freyhof.

Landry, L., W. F. Vincent, and L. Bernatchez. 2007. Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. Journal of Evolutionary Biology **20**:971-984.

Law, R. 2000. Fishing, selection, and phenotypic evolution. Ices Journal of Marine Science **57**:659-668.

Law, R. 2007. Fisheries-induced evolution: present status and future directions. Marine Ecology-Progress Series **335**:271-277.

Link, J., and M. H. Hoff. 1998. Relationships of Lake Herring (*Coregonus artedi*) gill raker characteristics to retention probabilities of zooplankton prey. Journal of Freshwater Ecology **13**:55-65.

Mertz, G., and R. A. Myers. 1998. A simplified formulation for fish production. Canadian Journal of Fisheries and Aquatic Sciences **55**:478-484.

Mookerji, N., C. Heller, H. J. Meng, H. R. Burgi, and R. Muller. 1998. Diel and seasonal patterns of food intake and prey selection by Coregonus sp. in re-oligotrophicated Lake Lucerne, Switzerland. Journal of Fish Biology **52**:443-457.

Müller, B., D. Finger, M. Sturm, V. Prasuhn, T. Haltmeier, P. Bossard, C. Hoyle, *et al.* 2007a. Present and past bio-available phosphorus budget in the ultra-oligotrophic Lake Brienz. Aquatic Sciences **69**:227-239.

Müller, R., M. Breitenstein, M. M. Bia, C. Rellstab, and A. Kirchhofer. 2007b. Bottom-up control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquatic Sciences **69**:271-288.

Myers, R. A., and J. M. Hoenig. 1997. Direct estimates of gear selectivity from multiple tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences **54**:1-9.

Nusslé, S., C. Bornand, and C. Wedekind. 2009. Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. Evolutionary Applications **2**:200-208.

Nusslé, S., A. Bréchon, and C. Wedekind. 2010. Change in individual growth rate and its link to gill-net fishing in two sympatric whitefish species. Evolutionary Ecology.

Nusslé, S., A. Bréchon, and C. Wedekind. in press. Change in individual growth rate and its link to gill-net fishing in two sympatric whitefish species. Evolutionary Ecology.

Opdal, A. F. 2010. Fisheries change spawning ground distribution of northeast Arctic cod. Biology letters **6**:261-264.

Palumbi, S. R. 2001. Evolution - Humans as the world's greatest evolutionary force. Science **293**:1786-1790.

Pigeon, D., A. Chouinard, and L. Bernatchez. 1997. Multiple modes of speciation involved in the parallel evolution of sympatric morphotypes of lake whitefish (Coregonus clupeaformis, Salmonidae). Evolution **51**:196-205.

R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ricker, W. E. 1981. Changes in the average size and average age of pacific salmon.

Canadian Journal of Fisheries and Aquatic Sciences **38**:1636-1656.

Rijnsdorp, A. 1993. Fisheries as a large-scale experiment on life-history evolution: disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia **96**:391-401.

Rijnsdorp, A. D., and F. Storbeck. 1995. Determining the onset of sexual maturity from otoliths of individual female North Sea plaice, *Pleuronectes platessa L.* In *Recent developments in fish otolith research*, edited by D. H. Secor, J. M. Dean and S. E. Campana: University of South Carolina Press, Columbia, S.C.

Roth, H., and W. Geiger. 1972. Brienzersee, Thunersee, and Bielersee - effects of exploitation and eutrophication on salmonid comunities. Journal of the Fisheries Research Board of Canada **29**:755-764.

Sharpe, D. M. T., and A. P. Hendry. 2009. Life history change in commercially exploited fish stocks: an analysis of trends across studies. Evolutionary Applications **2**:260-275.

Smith, T. B., and L. Bernatchez. 2008. Evolutionary change in human-altered environments. Molecular Ecology **17**:1-8.

Stearns, S. C. 1992. The evolution of life histories. New York: Oxford University Press.

Stokes, K., and R. Law. 2000. Fishing as an evolutionary force. Marine Ecology-Progress Series **208**:307-309.

Swain, D. P., A. F. Sinclair, and J. M. Hanson. 2007. Evolutionary response to size-selective mortality in an exploited fish population. Proceedings of the Royal Society of London B Biological Sciences **274**:1015-1022.

Theriault, V., D. Garant, L. Bernatchez, and J. J. Dodson. 2007. Heritability of life-

history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). Journal of Evolutionary Biology **20**:2266-2277.

Thomas, G., and R. Eckmann. 2007. The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth - the Lake Constance example revisited. Canadian Journal of Fisheries and Aquatic Sciences **64**:402-410.

Thomas, G., H. Quoss, J. Hartmann, and R. Eckmann. 2009. Human-induced changes in the reproductive traits of Lake Constance common whitefish (*Coregonus lavaretus*). Journal of Evolutionary Biology **22**:88-96.

Thorpe, J. E. 1998. Salmonid life-history evolution as a constraint on marine stock enhancement. Bulletin of Marine Science **62**:465-475.

Tolonen, A. 1998. Size-specific food selection and growth in benthic whitefish, *Coregonus lavaretus* (L.), in a subarctic lake. Boreal Environment Research **2**:387-399.

Vonlanthen, P., D. Roy, A. G. Hudson, C. R. Largiader, D. Bittner, and O. Seehausen. 2009. Divergence along a steep ecological gradient in lake whitefish (Coregonus sp.). Journal of Evolutionary Biology **22**:498-514.

Wright, R. F., A. Matter, M. Schweingruber, and U. Siegenthaler. 1981. Sedimentation in Lake Biel, an Eutrophic, Hard-Water Lake in Northwestern Switzerland. Schweizerische Zeitschrift Fur Hydrologie-Swiss Journal of Hydrology **42**:101-126.

Yoneda, M., and P. J. Wright. 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Marine Ecology-Progress Series **276**:237-248.

Table 1: Selection differential, adult growth decrease and mean generation time of five different species present in three different lakes.

Lake	Species	Selection differentials		Adult growth decrease		Gen. Time	
		Juv. growth	Ad. growth	per year	per gen.		
Joux	*C. palaea* (Palée)	NS	-4.9%	-0.94%	-4.39%	4.67 years	Nusslé et al. 2009
Brienz	*C. albula* (Brienzlig)	-3.9%	-17.4%	-2.02%	-7.71%	3.81 years	Nusslé et al. 2010
	C. fatioi (Albock)	NS	-7.0%	-2.71%	-9.72%	3.59 years	Nusslé et al. 2010
Biel	*C. confusus* (Bondelle)	-1.1%	-4.9%	-1.43%	-5.41%	3.79 years	this study
	C. palaea (Palée)	-1.1%	-4.8%	-1.95%	-7.34%	3.77 years	this study

Fig. 1: (A) Total phosphorus (mg/L) in Lake Biel (full circles), Lake Joux (empty circles) (Nusslé et al. 2009) and Lake Brienz (full triangles) (Nusslé *et al.* in press) during the last decades and (B) fishing yield (tons) in Lake Biel (full circles), Lake Joux (empty circles) (Nusslé et al. 2009) and Lake Brienz (full triangles) (Nusslé *et al.* in press)

Fig. 2: Growth parameters per cohort: (A-B) average juvenile growth (α_0) and (C-D) average adult growth (α_t). The lines represent regression lines (solid = significant linear relationship, dashed = non-significant).

Fig. 3: Selection differentials (*s*) estimated for each cohort. (A-B) *s* for juvenile growth (α_0), and (C-D) s for adult growth (α_t). The width of the circle corresponds to the number of fish within each cohort. The cohort is specified by the year of hatching from egg. The lines represent the average selection differential (solid = significantly different from zero, dashed = non-significant).

Fig. 1: Total phosphorus concentration and fishery yield in three alpine lakes.

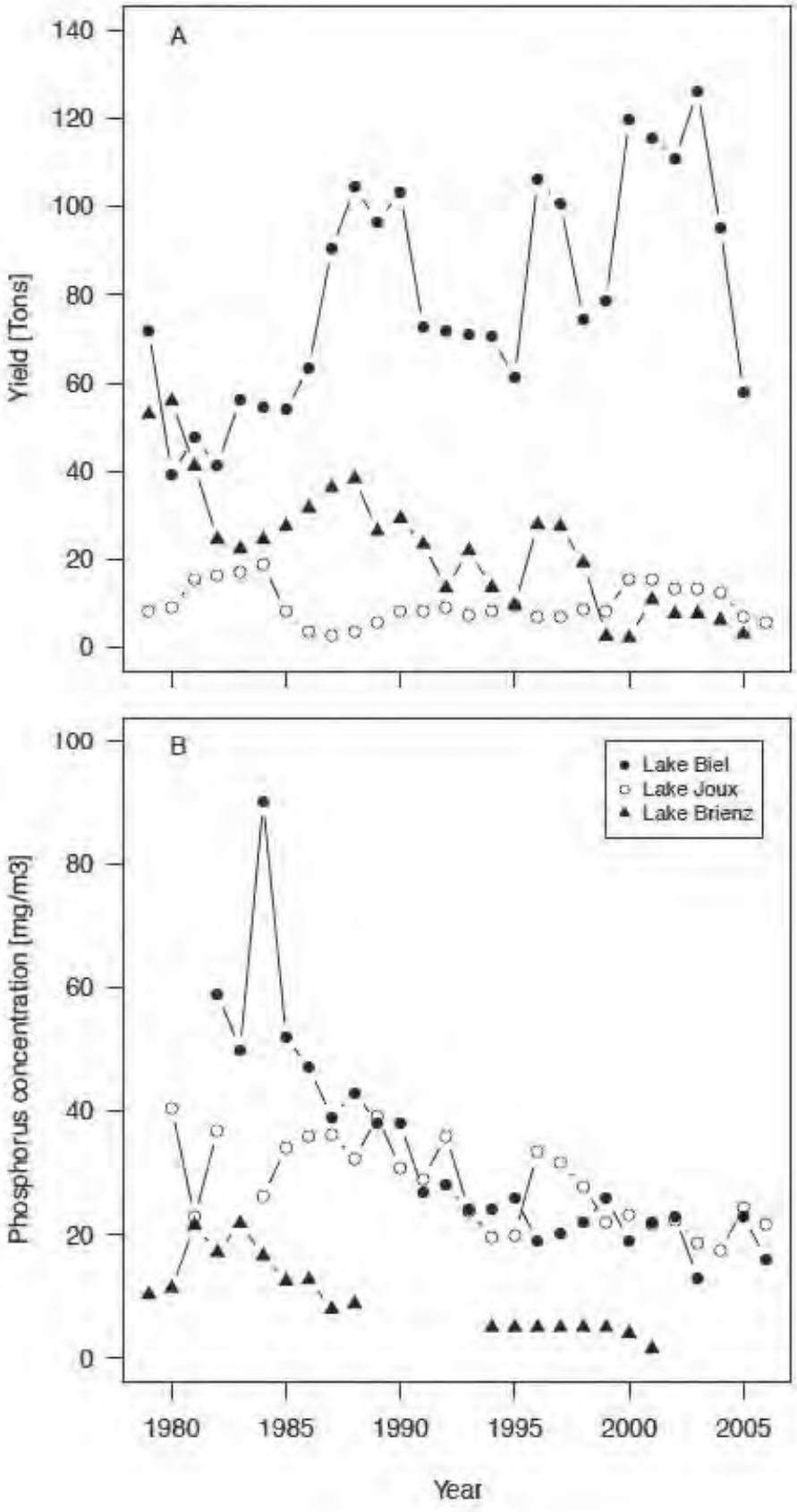


Fig. 2: Growth parameters over time

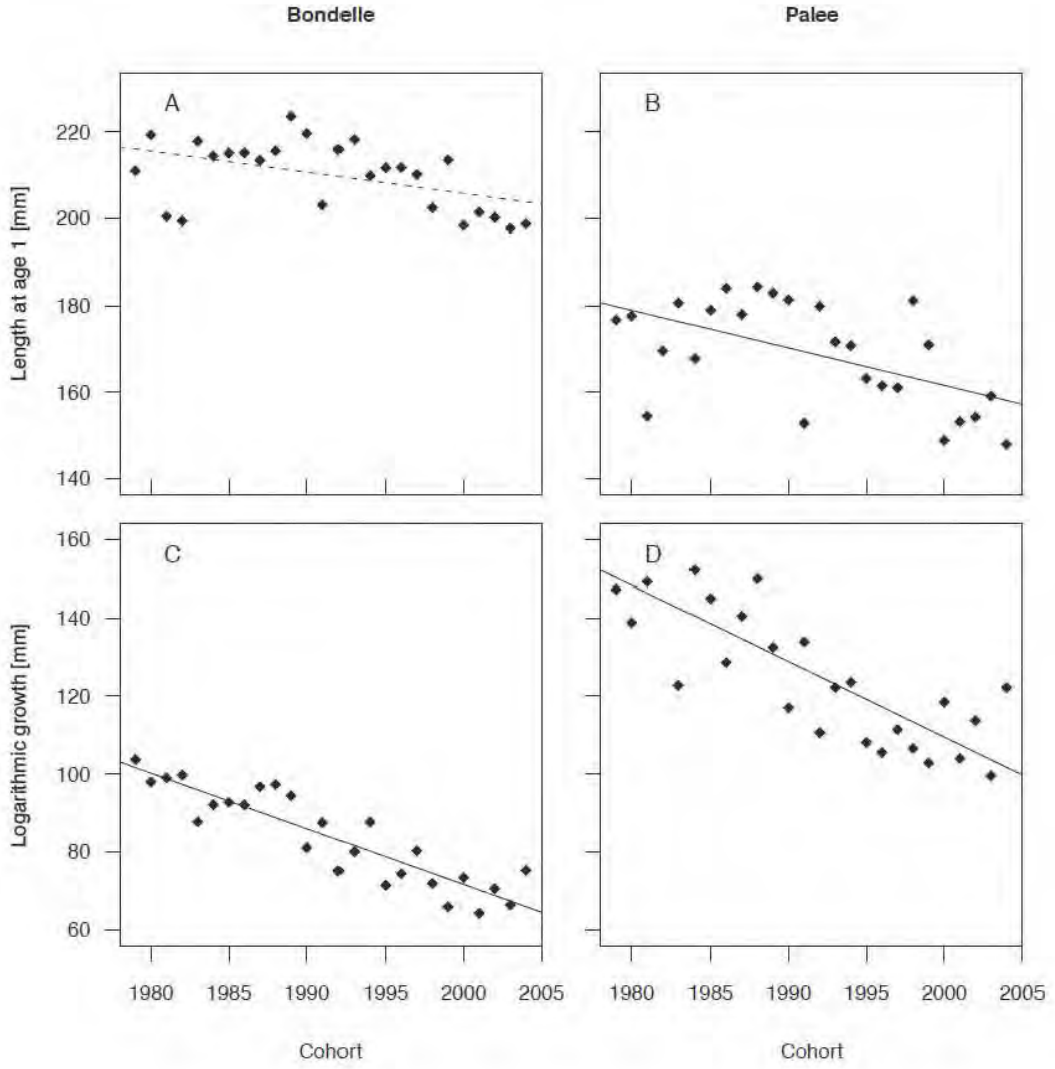
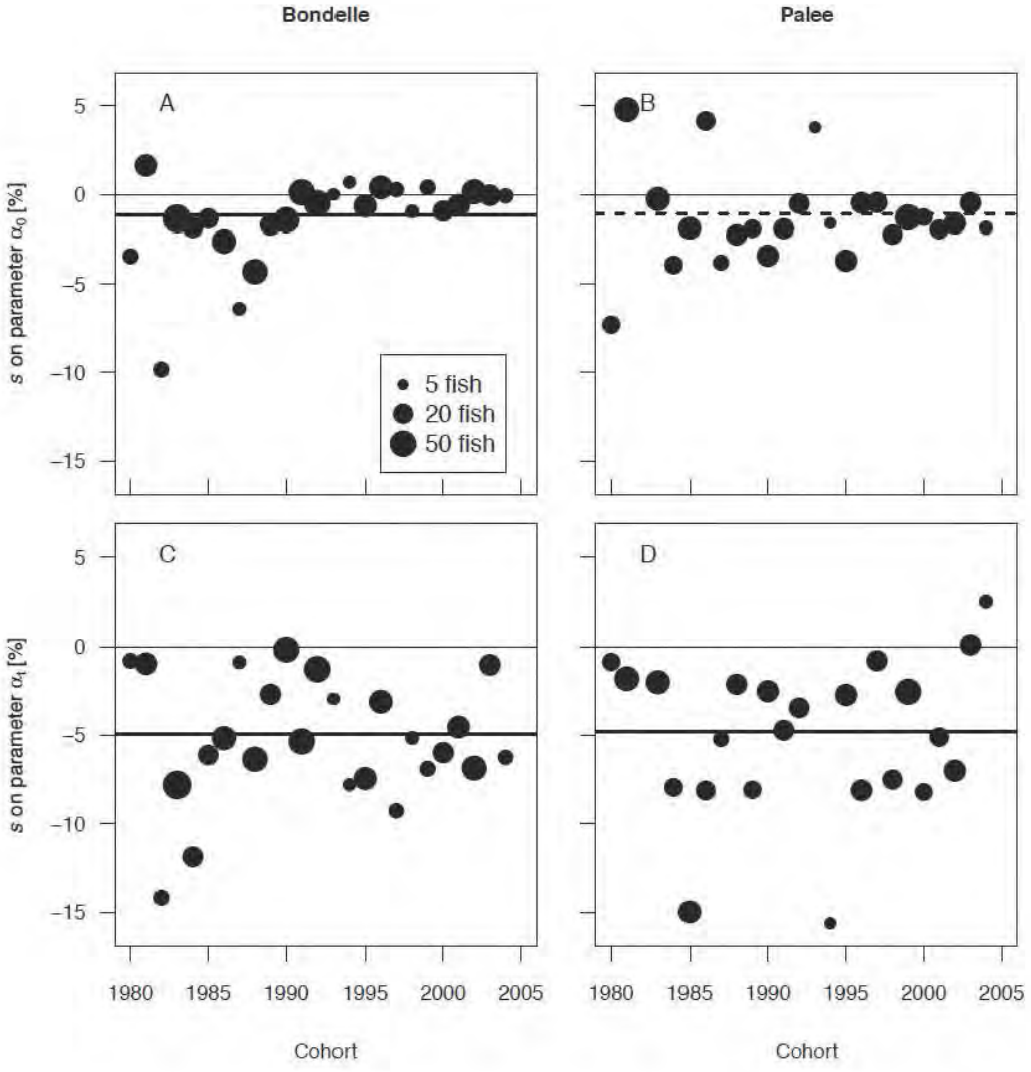


Fig. 3: Selection differentials (*s*) estimated for each cohort



Chapter 4

Towards reconciling fishery and fishery-induced evolution: predicting the effects of changed mesh size regulations

Sébastien Nusslé, Patrick Presi, Tadeus Kawecki, Claus Wedekind

Manuscript

Authors' contribution

SN and PP designed the model.
SN wrote the model and analysed the data.
SN, TK and CW discussed the study and the manuscript.
SN wrote the manuscript.

Remark following examination

A mistake in the model (chapter 4) was noticed during the examination. Phenotypic variance was mistakenly used for genetic variance in eq. 7 (page 89), and for generating environmental variance in the following. Preliminary simulations correcting these mistakes have been conducted since. They suggest that the general conclusion of the chapter is not affected, but only the magnitude of the effects. Hence, both the thesis director and the internal expert that noticed the mistake consider that the manuscript doesn't need to be modified at this stage (although a complete re-analysis will obviously be required for publication). Therefore, caution must be taken with regard to the magnitude of the effects reported in the current version of this chapter.

Towards reconciling fishery and fishery-induced evolution: predicting the effects of changed mesh size regulations

Running head: Fishery-induced evolution and fishing gear regulation

Sébastien Nusslé[§], Patrick Presi, Tadeus Kawecki, Claus Wedekind

Department of Ecology and Evolution, University of Lausanne, Biophore, 1015 Lausanne, Switzerland

§Corresponding author: snussle@gmail.com

Patrick Presi: patrick.presi@vphi.unibe.ch

Tadeus Kawecki: tadeus.kawecki@unil.ch

Claus Wedekind: claus.wedekind@unil.ch

Original research paper

Acknowledgments

We thank the *Service de Pêche du canton de Vaud* for providing the monitoring data, P. Amiet, C. Bornand, B. Büttiker, S. Guduff, F. Hofmann J.D. Meylan, Y. Meylan and C. Moresi for technical help and advice during data collection, E. Clark, M. dos Santos, R. Eckmann, G. Evanno, A. Jacob, N. Perrin, M. Pompini, A. Ross-Gillespie, B. von Siebenthal, R. Stelkens, T. Szekely Jr. and D. Urbach for discussion and/or comments on the manuscript, and the Swiss National Science Foundation and the Société Académique Vaudoise for financial support.

Abstract

Size-selective fishing can impose considerable selection pressure on life-history traits such as individual growth rate. The systematic removal of larger individuals is expected to have significant effects on the evolution of individual growth, on fishery yield, and potentially even on the persistence of harvested populations, but it is still not clear how management should best react. We studied the potential effects of various mesh-size regulations on an Alpine whitefish population using individual-based modelling. We found that certain changes in mesh-size regulations may not only prevent fishery-induced evolution of reduced growth, but may also help the population to recover from previous evolutionary effects of fishing. On the long run, mesh size regulations that avoid fishery-induced evolution on individual growth rates may even provide higher fishery yields than the current management regime. Our findings may help to reconcile short-term and long-term fishery interests with the demographic and genetic aspects of fish populations.

Keywords: whitefish, *Coregonus*, gillnet, fishery-induced adaptive change, evolutionary impact assessment, net selectivity, growth change

Introduction

Recent studies have demonstrated rapid phenotypic change due to harvesting in various kinds of organisms (Darimont *et al.* 2009). Phenotypic changes observed in 40 harvested populations were on average 300% more rapid than those observed in 20 non-harvested populations (Darimont *et al.* 2009). Moreover, the effect of harvesting on various phenotypic changes was on average 50% greater than the effects of any other anthropogenic factors that could be observed in 25 different populations (Darimont *et al.* 2009). Intense fishing is predicted to change life history traits such as age or size at maturation (Heino *et al.* 2002; Grift *et al.* 2003; Sharpe and Hendry 2009), the average reproductive effort (Yoneda and Wright 2004; Thomas *et al.* 2009), or individual growth rate (Handford *et al.* 1977; Ricker 1981; Thomas and Eckmann 2007; Nusslé *et al.* 2009). The relative contribution of ecological (plasticity) and evolutionary (genetic) factors on changes in life-history traits is still controversial (Hilborn 2006; Browman *et al.* 2008), but it seems obvious that any fishing technique that lead to a systematic removal of larger fish is likely to induce evolution. Experimental studies confirm this prediction (Conover and Munch 2002; Conover and Baumann 2009), and several populations have failed to recover, even after a complete fishing ban (Hutchings 2000; Hutchings and Reynolds 2004). See Jorgensen *et al.* (2007) for a review on fishing-induced changes of phenotypic traits. It thus becomes increasingly obvious that if we are to sustainably manage natural resources, we need to consider the impact of fishing on evolution (Stenseth and Dunlop 2009). Although fishery-induced evolution could dramatically change populations (Fenberg and Roy 2008), only few management plans have taken this problem into account (Stokes and Law 2000; Ashley *et al.* 2003; Smith and Bernatchez 2008) and it is still not clear how the management would best react to the situation. Predictive modelling

may help developing management scenarios that take into account the threat of fishery-induced evolution. A previous modelling study revealed that reduced fishing efforts on brook charr (*Salvelinus fontinalis*) may be beneficial not only for the long-term conservation of populations by avoiding harvest-induced evolutionary changes, but that this moderation may also offer economical advantages for fisheries, i.e. by securing yield in the near future (Okamoto *et al.* 2009). Simulation studies in Northeast Arctic cod (*Gadus morhua*) have shown that employing bell-shaped size selective fishing gear (such as gillnets) rather than sigmoid size-selective (such as trawling) would favour both slow and fast growers (Jorgensen *et al.* 2009). Another theoretical study on Atlantic cod (*Gadus morhua*) has modelled the effectiveness of implementing marine reserves to reduce and reverse the evolutionary impact of fishing, and the impact of such management scenarios on fishing yield (Dunlop *et al.* 2009).

Here, we use individual-based modelling to predict the evolutionary consequences of different size-selective fishing gear. We based our analyses on the 30-year long monitoring of an isolated and well-defined population of the whitefish *Coregonus palaea* that is under high but rather constant (and well-documented) fishing pressure. A previous study on this population found significant changes in individual growth rates that could be linked to gill-net fishery (Nusslé *et al.* 2009), but no consistent changes in the timing of maturation over the observational period. We therefore concentrated our analyses on the likely evolution of juvenile and adult growth rates under various management scenarios. We found that fishing within a certain length window may not only prevent fishery-induced evolution of growth but also help the harvested population to recover from previous deleterious effects of fishing.

Methods

The study population

Our model is based on empirical data taken from a population of the Alpine whitefish *Coregonus palaea* of Lake Joux, Switzerland (46.63°N, 6.28°E, 9.5 km^2, maximum depth = 32 m). This population has been monitored in the course of a state program since 1980. So far, 2,272 fish have been sampled and measured for length and weight. A majority of them were also sexed. Scale samples were taken for length-at-age determination. The study population reproduces once a year in December. Maturation occurs at an average (±SD) age of 3.1 ± 0.8 years in both sexes. Age at maturation was calculated according to Rijnsdorp *et al.* (1995) (for details see Nusslé *et al.* (2009). Maximum age in these samples was 13 years, but most fish are harvested at the age of four years. Length back-calculations, calculated weight-at-age, and the proportion of mature individuals are reported in Table 1. To estimate total adult mortality in the lake (fishing plus natural mortality) and the age structure we focused on the part of the population that experienced both fishing and natural mortality. Total mortality was estimated as the slope of the regression line between log-transformed number of sampled fish (dependent variable) and their age (explaining variable). Only individuals between 4 and 10 years of age were used in these calculations. Three-year-old fish are often too small to be caught by professional fishing gear and therefore do not experience full fishing mortality. Fish older than 10 years were excluded because they represented less than 1% of the catches but may have a disproportionately large impact on calculations due to leverage effects. Moreover, age determination is increasingly less accurate with increasing age of the fish.

Individual-based model

The purpose of our model is to assess the expected change of individual growth (juvenile and adult growth) in the study population and under different management scenarios, i.e. using different size-selective fishing gear combinations. Our model is multi-generational and tracks the simulated population at annual time steps. Since the sex ratio seems stable over time and not significantly differ from 1:1 in our study population (Naceur and Büttiker 1999; Nusslé *et al.* 2009), we only modelled the dynamics of females. Individual-level variables included: age (years), modelled genotypes for juvenile and adult growth that result in realized phenotypes of juvenile and adult growth, length (mm), weight (g), cohort (year of birth), and survival to the next time period (yes or no).

Individual growth rates were dependent on stochastic processes and heritable growth parameters. The length of individuals was modelled on the basis of the two-parameters growth model described in Nusslé *et al.* (2009), where the fish length *L(t)* is a function of fish age t, of the phenotypic juvenile growth (α_{p0}), and of the phenotypic adult growth (α_{pt}).

$$L(t) = \alpha_{p0} + \alpha_{pt} \log(t) \quad (1)$$

Fish weight *W* was estimated from the following allometric relationship to fish length:

$$W = aL^b \quad (2)$$

The coefficients of the relationship were assessed with a log-log regression on a total of 1,338 individuals (for which both weight and length measurements were available) and set to $a = 6.1 \times 10^{-6}$ and $b = 3.05$.

Natural mortality was assumed to be random, constant, and independent of the total biomass in the lake (density dependence is taken into account during the recruitment phase, see below).

Fishing mortality was defined as the multiplication of two different factors: the gillnet encounter rate during a year (which is the probability for a fish to encounter a fishing gear during one whole year and that only depends on fishing intensity), and the probability for a fish to get caught by the net if encountered (which is defined as a selectivity function). The most commonly used selectivity functions in the literature for gill-net fishing are Gaussian curves (Jensen and Hesthagen 1996; Jorgensen *et al.* 2009; Kuparinen *et al.* 2009) with several skewed variants (Jensen and Hesthagen 1996; Millar and Fryer 1999) to account for the skew towards larger fish observed in the wild (Reis and Pawson 1999; Fukuwaka *et al.* 2008). Although this traditional modelling scheme works for general hypotheses (Jorgensen *et al.* 2009), it may sometimes be insufficient if selectivity is not maximal for a certain modal length or does not decrease continuously as fish length differs from this modal length. Indeed, some empirical data of gillnet selectivity show a length distribution that differs from a normal distribution (Jensen and Hesthagen 1996; Reis and Pawson 1999; Prchalova *et al.* 2009). Selectivity distributions are often flatter than a Gaussian curve, i.e. fish within a certain length window seem to have an equal probability of being caught (Hamley 1975). Moreover, gillnet selectivity is more related to fish girth than to fish size (Reis and Pawson 1999). While girth is proportional to length, the variance found in girth is expected to be smaller than the variance in length as length measurements cover a much larger range than girth measurements. It is therefore likely that fish with the same girth have different lengths. Furthermore, fish of same length can get entangled at varying positions (close to the mouth up to behind the dorsal fin; see (Reis and Pawson 1999). In general, there are three different ways in which a fish can be caught in a gillnet (Hamley 1975): wedged (held tightly by a mesh around the body), gilled (prevented from backing out of the net by a mesh

caught behind the gill cover), or tangled (held in the net by teeth, maxillaries or other projections). The proportion of fish caught in each of these three ways varies between species (Reis and Pawson 1999). It has been shown that the left slope of the selectivity curve represents to a large degree small fish wedged in the net while the right slope represents rather tangled large fish. This might result in skewed or even multimodal curves (Hamley 1975). Finally, gillnets are unable to enmesh fish smaller than a certain size limit because individuals whose girth is smaller than the mesh diameter can swim through the net (Hamley 1975; Prchalova *et al.* 2009). For all these reasons, we believe that a smooth Gaussian curve, even skewed, can be a too strong assumption for our model, particularly if size-selectivity leads to adaptive change. A Gaussian selectivity function where even a slight deviation from the modal length is advantageous may drive evolution more quickly than a flat selectivity function where all individuals within a certain size window are equally likely to be caught.

We tested two different selectivity functions in order to account for the above mentioned potential pitfalls: a traditional Gaussian function and a table-like function (Hamley 1975). These function were then compared to the actual catch data from our study population (Figure 1). Reference populations were modelled with an age structure based on natural and fishing mortality, and length-at-age distributions were based on back-calculations from scales (Nusslé *et al.* 2009). To account for variability we used a Bayesian approach employing model parameters with known distributions (as opposed to fixed parameters). We created 10,000 reference populations in which the proportion of fish per age class was inferred assuming a certain natural mortality (a random number drawn from a uniform distribution between 20% and 50% mortality) and a certain gillnet encounter rate (a random number drawn from a

uniform distribution between 30% and 60% of the remaining fish). We assumed (and the actual catch data suggest) that only half of the three year old fish were harvested, and that individuals younger than three years experienced only natural mortality. Once natural and fishing mortalities had acted on our model populations, the length of individual survivors was determined as a random number drawn from a normal distribution with mean and variance estimated from length-at-age back-calculations (Table 1).

Both the Gaussian function and the table-like function were applied to our reference populations and then compared to the actual catch data (Figure 1). With the Gaussian function we assumed that the probability of being selected peaks at a certain modal length, which is equal to the mean length of the observed catch plus its standard deviation multiplied by a factor (*mult1*) taken from a uniform distribution between -0.5 and 1.5. This takes into account that the true modal length may be smaller or larger than the observed modal length. The probability of being selected decreases following a Gaussian curve with a mean equalling the modal length and the standard deviation equalling the standard deviation of the observed catch times another random number (*mult2*) taken from a uniform distribution between 0.5 and 2. This takes into account that the true standard deviation of the selectivity curve may be smaller or larger than the observed standard deviation. In the table-like function we assumed that the selectivity function is flat and maximal between two intermediate length values (*low* and *high*), and then linearly decreases to extreme length values (*min* and *max*). *Min* and *max* are the limit of the length distribution beyond which fish cannot be harvested (the selectivity of the net are null; because the fish are too small or too large to get entangled). These extremes values where randomly sampled within the percentile 0% and 0.5% of the observed length distribution of the catch for *min*

and between the percentiles 99.5% and 100% for *max*. *Low* and *high* values were also sampled within the length distribution of the catch between *min* and the median for *low* and the median and *max* for *high*. 10,000 replications were performed in order to find the best parameter set, defined as the set that minimizes the absolute difference between the observed and the modelled length distributions. These comparisons of length distributions were based on histograms on 20 mm length intervals between zero and 860 mm. A second run of parameter selection was then performed, based on the previous results, in order to increase the precision of the parameters estimation.

Reproduction occurred at the end of each annual cycle if a simulated population contained at least one mature female. For simplicity, and because previous observations suggest that the age at maturation does not change over time (Nusslé *et al.* 2009), maturation in the simulations was set at the age of three years, without potential for evolution. The new generation enters the population as one-year-old fish at the beginning of the next annual cycle. The number of offspring produced each year was derived from a modified Ricker's model (Ricker 1954; Haddon 2001): the number of individuals in Ricker's model was replaced by total biomass (kg) to mimic density-dependence and intraspecific competition between adults and juveniles (Naceur and Büttiker 1999). The recruited biomass in the lake (B_R) was assumed to be a function of the stock's biomass (B_S), the recruited biomass rate in absence of competition (r_S) and the rate of decrease of recruited biomass as *Bs* increases (d). r_S was set to 1 and d was set to 0.001.

$$B_R = r_S B_S e^{-dB_S} \quad (4)$$

We then transformed the recruited biomass into number of offspring. The number of offspring produced (N_{off}) is equal to the recruited biomass B_R divided by the average weight of one-year-old fish $\overline{w_1}$, calculated from equation (2).

$$N_{off} = \frac{B_R}{\overline{w}_1} \quad (5)$$

Once the total number of offspring was calculated (N_{off}), each female was assigned a random number of offspring. This number was taken from a Poisson distribution of parameter λ_i, which depends on the number of reproducers (N_{rep}), the length of each reproducing female (L_i) and the average length of reproducing females ($\overline{L}$):

$$\lambda_i = \frac{N_{off}}{N_{rep}}\left(\frac{L_i}{\overline{L}}\right) \quad (6)$$

This equation takes the higher fecundity of large females into account, but also the potentially higher survival of offspring from large females due to maternal investment (Birkeland and Dayton 2005).

The genetic growth parameters (α_{gj}) were assumed to represent the additive effects of several loci. Each offspring's genetic growth parameter was calculated as the average of the respective maternal growth parameter ($\alpha_{gj,mother}$, where *j* equals *0* or *t*) and paternal growth parameter. The latter was a random number taken from a normal distribution with a mean equal to the population mean after selection ($\alpha_{gj,pop}$), and a variance equal to the phenotypic variance in the population (V_P).

$$\alpha_{gj} = \frac{\alpha_{gj,mother} + N\left(\alpha_{gj,pop}, V_P\right)}{2} \quad (7)$$

Phenotypic growth (α_{pj}) was calculated by adding a random number to genetic growth (α_{gj}). This random number was taken from a normal distribution with a mean of zero and a variance equal to phenotypic variance (V_P) multiplied by the heritability (h^2) of juvenile and adult growth. This heritability was first set to 0.3, i.e. an intermediate value for life-history traits in salmonids (Law 2000; Garcia de Leaniz *et al.* 2007; Swain *et al.* 2007; Theriault *et al.* 2007; Carlson and Seamons 2008).

$$\alpha_{pj} = \alpha_{gj} + N\left(0, h^2 V_P\right) \quad (8)$$

Because the heritability of juvenile growth may be smaller than the heritability of adult growth, and because heritability estimates can be highly variable, we computed sensitivity analyses with heritability estimates varying from 0.15 to 0.45 for adult growth and from 0.05 to 0.35 for juvenile growth.

We tested the potential effects of three different management scenarios: (1) continuing with the current management (a combination of 45 and 50 mm gill net mesh sizes), (2) a second management scenario allowing for 45 mm mesh size only, and (3) a third management scenario allowing for 40 mm mesh size only.

Calibration

Model parameters were calibrated to the empirical data inferred from the monitoring program in the following manner. We ran the model under the current management scenario (i.e. with mesh sizes of 45 and 50 mm) for 25 years and for all possible combinations of parameters. Gillnet encounter rate varied from 30% to 60% (with steps of 5%), natural mortality varied from 20% to 50% (with steps of 5%). Individual growth parameters in the natural population were equal to 123 ± 20 mm for phenotypic juvenile growth (α_{p0}) and 171 ± 31 mm for adult growth (α_{pt}) (mean ± SD). Then, we fixed genotypic juvenile growth to 125 mm, and varied genotypic adult growth from 140 to 200 mm (with steps of 5 mm). In total 637 different parameter sets were run for each selectivity function. We then computed the age distribution, i.e. the proportion of fish within each age class, and the size at age distribution, i.e. the average length in each age class, for every parameter combination. For each combination and for each age class, we calculated the absolute difference between the modelled value and the observed value and used the sum of

these absolute differences to assess the accuracy of the model estimates We selected the parameter set that minimized the differences in both the age and the length distribution.

Sensitivity analyses

To assess the sensitivity of our parameter estimates, 5,000 replicates for each of the three potential management scenarios that we investigated (i.e. three different mesh size regulations) and for each net selectivity function (Gaussian and table-like) were repeated with changing values for the various key parameters (fishing and natural mortalities, heritability and initial values for juvenile and adult growth). All parameters values were sampled from uniform distributions within the ranges that are listed in Table 2.

Results

Model inputs

With regard to the net selectivity for the Gaussian and the table-like function, respectively, the best fit between model and observation is achieved with the following parameter set: mult1 = 0.25, mult2 = 1.25, min = 0.1%, max = 99.9%, low = 35% and high = 95%. The parameters that maximize convergence between modelled and observed age and length distributions (Figure 2) depend on the net selectivity. The best parameter set for the table-like function was found to be a combination of gillnet encounter rate (40%), natural mortality (30%), juvenile growth (125 mm), and adult growth (165 mm). The best parameter set for the Gaussian function closely resembled the optimal set for the table-like function, with gillnet encounter rate (40%), natural mortality (35%), juvenile growth (125 mm), and adult growth (160 mm). The fit between simulations and observations for the table-like

function is plotted in Figure 2 (see Supplementary Figure 1 for the best fitting Gaussian function).

Model outputs

When using the best parameter set for both the table-like function and the Gaussian function, we found that the expected change in growth rate were similar for both selectivity functions, with the table-like function giving slightly more pessimistic outlooks (Figure 3, Supplementary Figure 2). Based on the table-like function and assuming the current management scenario, i.e. a gillnet encounter rate of 40% with 45 and 50 mm mesh size nets and 30% natural mortality (respectively 35% for the Gaussian function), the average (±SD) yearly change in juvenile growth would be -0.068 ± 0.006% per year. For adult growth, yearly changes of -0.101 ± 0.015% are expected. With the Gaussian function the expected change would be equal to -0.051 ± 0.009% for juvenile growth and -0.08 ± 0.015% for adult growth. Under the third management scenario (40 mm mesh size only), we expect almost no change in juvenile growth: 0.007± 0.010% per year (Gaussian function: -0.005 ± 0.010%) but an increased adult growth of 0.082 ± 0.0077% per year (Gaussian function: -0.047 ± 0.012%). The changes in growth expected under the second management scenario (45 mm mesh size) lie between these two extremes (Figure 3).

Under the current management scenario the total yield per year is expected to decrease over time from around 500 kg to less than 400 kg in a few decades (Figure 4a). With the second management scenario, yield is expected to be reduced due to the management measure but should remain relatively stable around 450 kg and may even increase slightly eventually. With the third management scenario, yield is expected to drop to around 300 kg and then continue to decrease. Results are similar with the Gaussian selectivity function, except that it may take longer for the yield

under the second management scenario to catch up with the yield under the current management scenario.

Sensitivity analysis

The expected decrease expected for adult growth with the sensitivity analyses is similar with the two different selectivity functions (Gaussian and table-like) for each of the three management scenarios (Table 3). We therefore pooled the 5'000 replications performed with each selectivity function to assess the total sensitivity of our estimates (Figure 5).

Assuming the current management scenario, the average (±SD) yearly change in juvenile growth would be -0.069 ± 0.026% per year, and for adult growth: yearly changes of -0.112 ± 0.060% are expected. Assuming the second management scenario, changes of are expected for juvenile growth and -0.036 ± 0.056% for adult growth. Under the third management scenario, we expect almost no change in juvenile growth: -0.010 ± 0.022% per year but an increased adult growth of 0.045 ± 0.044% per year.

Discussion

We used data from a well defined long-term monitoring program on Alpine whitefish from a small lake in Switzerland (Nusslé *et al.* 2009) that runs since 1980. We simulated fishery-induced changes in growth rates with individual-based models, and tested alternative management scenarios that would allow for both the conservation of individual growth rates and the preservation of future yields. Current management regulations allow for combinations of nets with mesh sizes of 45 mm and 50 mm (Naceur and Büttiker 1999). In the event of decreasing average fish size in the professional catches, the traditional management measure in this region is to

reduce the minimal authorized mesh size in order to catch smaller fish (Naceur and Büttiker 1999). Our model predicts that, if the currently applied harvesting technique is retained, and if the environment remains constant, a fishery-induced, adult growth decrease of ca. 0.1%, per year is to be expected (Figure 2, Figure 5, Table 3). In a previous study (Nusslé *et al.* 2009), we found that the real growth decrease observed in this population is around one percent per year (0.97 ± 0.36 %), including both environmental effects and fishery-induced selection. If our model is correct, this indicates that ca. 10% (0.1 / 0.97) of the change in growth observed in the wild can be attributed to the impact of size-selective fishing on the genetic composition of the population. The contribution of harvesting found in this study is in the same order of magnitude, however slightly lower. In the study mentioned above, where we measured the selection differential of fishing on growth parameters, we found that about 30 % of the decrease may be directly linked to size-selective fishing (Nusslé *et al.* 2009). Our model further supports the hypothesis that the systematic removal of larger individuals by fishing can exert substantial selection on life-history traits including individual growth rate.

If the average body size decreases due to fisheries-induced selection, fish will be caught at an older age, and more fish will die of natural causes before being recruited by fisheries. Moreover, the fish caught will become smaller over time, leading to fewer fish being harvested because most individuals will likely be too small for the mesh sizes used. Overall, yield is therefore expected to decrease (Figure 4). The long-term perspective of the current fishing regulations resembles an evolutionary dead-end where fast growers are at a disadvantage, ultimately leading to a collapse of the population, as seen with some marine populations that fail to recover, even after cessation of fishing (Hutchings 2000; Hutchings and Reynolds

2004). The disconcerting challenge is that natural selection driving the recovery of genetic variation of life-history traits is weaker than selection exerted by harvest. It may therefore take a long time until the population will have re-established itself to pre-harvest levels (Conover *et al.* 2009; Enberg *et al.* 2009). Another potential outcome of the current harvesting regime may be the complete avoidance of being fished, i.e. the population may evolve to contain mainly slow growers or alternative strategies to avoid fishing-induced mortality would evolve, such as specific morphological adaptations (Heino and Godo 2002). Regardless of the selectivity function, fishing yields would most likely diminish over the years.

From an ecological viewpoint, several consequences may arise from size-selective harvesting (Fenberg and Roy 2008). Despite controversies with regard to the imminent consequences of removing large fish (Carlson *et al.* 2008), there is broad consensus that we need to develop a precautionary approach to managing evolving fish stocks (Francis and Shotton 1997) for the following reasons. First, growth is energetically costly and fast growers are likely to have a higher reproductive success than small and slow-growing individuals (Birkeland and Dayton 2005). A systematic removal of 'high quality' adults could therefore result in an increase of the population's genetic load. Second, large females usually have higher fecundity and often produce larger offspring of higher viability (Trippel 1995; Walsh *et al.* 2006). A decrease in growth results in smaller females which can impair their reproductive success and consequently the long-term yield of the population. Third, females of many fish species have a mating preference for large males (Hutchings and Rowe 2008) and an increased mortality of large fish may impact the evolution of mate choice and alter the sexual selection regime of a population. Fourth, the depletion of larger fish might be have unpredictable effect to the entire ecosystem, given that fish

have a differential impact on the ecosystem depending on their size (Bruggemann *et al.* 1996; Friedlander and DeMartini 2002). Finally, non-random mortality can decrease the genetic diversity of a population and may render it more vulnerable to environmental stress such as temperature increase or epidemics (Jones *et al.* 2001). All these detrimental effects of selective harvest can potentially be reduced by protecting larger individuals and thus preserving the population's natural size distribution and genetic diversity for life history traits as proposed in this study.

Our data show that implementing a relatively simple management scenario, i.e. a reduced catch-range of body sizes, could allow fast growers to escape fishery-induced selection, and eventually help achieve two desirable long-term goals. Not only could harvesting with nets of different mesh sizes decelerate the observed decrease in growth, but it may also lead to a complete reversal of the trend, ultimately causing an increase in the average growth rate of the individuals. This management action could counteract previous deleterious effects of fishing on the genetic variability of growth in the population. A conservation focus on protecting larger (and thus older) individuals has been recommended before as larger individuals favour recruitment due to higher survival and competitiveness of their offspring (Birkeland and Dayton 2005). Our model does not take into account the genetic diversity that may have already been lost over several decades of harvesting, and our hypothesis may hold only if sufficient diversity remains in the lake. It has indeed been shown empirically that a genetically based reversal of fishing-induced evolution was possible if sufficient genetic variation in the population is present (Conover *et al.* 2009). The large variance in growth observed in our study population suggests that a recovery of the population may be possible.

With regards to yield, any restriction on nets with larger mesh size would result in lower yield due to the size selectivity of the nets (Figure 4). The use of a reduced size window for harvesting implies that fewer fish would be caught and that these fish would be smaller, which is not in favour for the local fishing industry. However, our model predicts that the implementation of a maximal mesh size could ensure the sustainability of the population and fishing yields in a population managed in this way may eventually surpass the yield of a population kept under the current management scenario (Figure 4C). Our model also predicts that changing management scenario have to be implemented with care, since reducing the size window too drastically could result in decreasing yield (Figure 4B). This is probably due to growth rates sufficiently high to escape the fishing window quickly enough to avoid complete harvesting. Another challenge for finding the right size window is the risk of increasing fishing-induced mortality. If fast growers are prevented from reaching a size larger than the maximal mesh size, we would expect a dramatic drop in growth rate (because small fish would be caught while, at the same time, large fish would not be favoured). This pattern is demonstrated in Figure 3, where strong fishing pressure induces dramatic decreases in both adult and juvenile growth.

Interestingly, our model predicts that juvenile growth should decrease over time, but this has not been confirmed in the empirical data set where juvenile growth remained constant (Nusslé *et al.* 2009). Indeed, small juvenile fish may attain a smaller adult size than their larger conspecifics, and thus suffer less from fishing-related selection. Moreover, juvenile and adult growth rates are likely to be genetically correlated (Lande and Arnold 1983; Walsh *et al.* 2006) and selection on adult growth should therefore indirectly affect juvenile growth. A possible explanation for the absence of growth decrease observed in the wild population may

be that juvenile growth, a single length-at-age measure, is probably more strongly influenced by environmental factors than adult growth, which is averaged over several years and may therefore be more stable with regard to environmental variation. Temperature is known to have a significant impact on juvenile growth in that it increases the metabolic rate and therefore the growth (Malzahn *et al.* 2003; Coleman and Fausch 2007; Gunther *et al.* 2007). It is thus possible that the genetically based decrease in growth may be masked by a plastic response to warmer temperatures in Swiss lakes and rivers that have been recorded over the last decades. If the environment impacts juvenile growth more strongly than adult growth, heritability for juvenile growth should be reduced. We computed sensitivity analyses with reduced heritability for juvenile growth, but found no significant influence of heritability on juvenile growth, indicating that fishing-induced selection mainly acts on adult growth. Another explanation for this lack of change in juvenile growth in the wild population could be that the constant growth rate observed in juveniles is an adaptive response to resource allocation, with more energy invested into juvenile growth and survival and less energy into adult growth.

Fishery-induced evolution impacts fish populations and this effect is likely substantial. Our findings highlight the tightly entangled interactions between selection and environment on the characteristics of fish populations and our results stress the importance of taking both ecological and economical factors into account when establishing management plans. Simple measures, such as fishing gear adjustment, could be beneficial to all stakeholders. Such regulations should, however, be implemented with care. A thorough risk assessment of fishing gear selectivity, of the age structure and length-at-age distribution, and a quantification of both fishing and natural mortality is required to avoid potentially dramatic consequences. This might

require several years of monitoring, as populations are likely to fluctuate from year to year, and a strict monitoring following the changes in regulations.

Bibliography

Ashley, M. V., M. F. Wilson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation **111**:115-123.

Birkeland, C., and P. K. Dayton. 2005. The importance in fishery management of leaving the big ones. Trends in Ecology & Evolution **20**:356-358.

Browman, H. I., R. Law, Marshall, C. Tara, A. Kuparinen, Merila, Juha, *et al.* 2008. The Role of Fisheries-Induced Evolution. Science **320**:47b-50.

Bruggemann, J. H., A. M. vanKessel, J. M. vanRooij, and A. M. Breeman. 1996. Bioerosion and sediment ingestion by the Caribbean parrotfish *Scarus vetula* and *Sparisoma viride*: Implications of fish size, feeding mode and habitat use. Marine Ecology-Progress Series **134**:59-71.

Carlson, S. M., E. M. Olsen, and L. A. Vollestad. 2008. Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. Functional Ecology **22**:663-673.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications **1**:222-238.

Coleman, M. A., and K. D. Fausch. 2007. Cold summer temperature regimes cause a recruitment bottleneck in age-0 Colorado River cutthroat trout reared in laboratory streams. Transactions of the American Fisheries Society **136**:639-654.

Conover, D. O., and H. Baumann. 2009. The role of experiments in understanding fishery-induced evolution. Evolutionary Applications **2**:276-290.

Conover, D. O., and S. B. Munch. 2002. Sustaining fisheries yields over evolutionary time scales. Science **297**:94-96.

Conover, D. O., S. B. Munch, and S. A. Arnott. 2009. Reversal of evolutionary downsizing caused by selective harvest of large fish. Proceedings of the Royal Society B-Biological Sciences **276**:2015-2020.

Darimont, C. T., S. M. Carlson, M. T. Kinnison, P. C. Paquet, T. E. Reimchen, and C. C. Wilmers. 2009. Human predators outpace other agents of trait change in the wild. Proceedings of the National Academy of Sciences of the United States of America **106**:952-954.

Dunlop, E. S., M. L. Baskett, M. Heino, and U. Dieckmann. 2009. Propensity of marine reserves to reduce the evolutionary effects of fishing in a migratory species. Evolutionary Applications **2**:371-393.

Enberg, K., C. Jørgensen, E. S. Dunlop, M. Heino, and U. Dieckmann. 2009. Implications of fisheries-induced evolution for stock rebuilding and recovery. Evolutionary Applications **2**:394-414.

Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209-220.

Francis, R. I. C. C., and R. Shotton. 1997. "Risk" in fisheries management: a review. Canadian Journal of Fisheries and Aquatic Sciences **54**:1699-1715.

Friedlander, A. M., and E. E. DeMartini. 2002. Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian islands: the effects of fishing down apex predators. Marine Ecology-Progress Series **230**:253-264.

Fukuwaka, M. A., T. Azumaya, N. D. Davis, and T. Nagasawa. 2008. Bias in size composition of chum salmon (*Oncorhynchus keta*) caught by a gillnet with a

geometric series of mesh sizes, and its correction using gear intercalibration. Ices Journal of Marine Science **65**:930-936.

Garcia de Leaniz, C., I. A. Fleming, S. Einum, E. Verspoor, W. C. Jordan, S. Consuegra, N. Aubin-Horth, *et al.* 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews **82**:173-211.

Grift, R. E., A. D. Rijnsdorp, S. Barot, M. Heino, and U. Dieckmann. 2003. Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Marine Ecology-Progress Series **257**:247-257.

Gunther, S. J., R. D. Moccia, and D. P. Bureau. 2007. Patterns of growth and nutrient deposition in lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*) and their hybrid, F-1 splake (*Salvelinus namaycush* x *Salvelinus fontinalis*) as a function of water temperature. Aquaculture Nutrition **13**:230-239.

Haddon, M. 2001. Modelling and quantitative methods in fisheries. Boca Raton, Florida: Chapmann & Hall/CRC.

Hamley, J. M. 1975. Review of Gillnet Selectivity. Journal of the Fisheries Research Board of Canada **32**:1943-1969.

Handford, P., G. Bell, and T. Reimchen. 1977. Gillnet fishery considered as an experiment in artificial selection. Journal of the Fisheries Research Board of Canada **34**:954-961.

Heino, M., U. Dieckmann, and O. R. Godo. 2002. Estimating reaction norms for age and size at maturation with reconstructed immature size distributions: a new technique illustrated by application to Northeast Arctic cod. Ices Journal of Marine Science **59**:562-575.

Heino, M., and O. Godo. 2002. Fisheries-induced selection pressures in the context of sustainable fisheries. Bulletin of Marine Science **70**:639-656.

Hilborn, R. 2006. Faith-based fisheries. Fisheries **31**:554-555.

Hutchings, J. A. 2000. Collapse and recovery of marine fishes. Nature **406**:882-885.

Hutchings, J. A., and J. D. Reynolds. 2004. Marine fish population collapses: Consequences for recovery and extinction risk. Bioscience **54**:297-309.

Hutchings, J. A., and S. Rowe. 2008. Consequences of sexual selection for fisheries-induced evolution: an exploratory analysis. Evolutionary Applications **1**:129-136.

Jensen, J. W., and T. Hesthagen. 1996. Direct estimates of the selectivity of a multimesh and a series of single gillnets for brown trout. Journal of Fish Biology **49**:33-40.

Jones, M. W., T. L. McParland, J. A. Hutchings, and R. G. Danzmann. 2001. Low genetic variability in lake populations of brook trout (*Salvelinus fontinalis*): A consequence of exploitation? Conservation Genetics **2**:245-256.

Jorgensen, C., K. Enberg, E. S. Dunlop, R. Arlinghaus, D. S. Boukal, K. Brander, B. Ernande, *et al.* 2007. Ecology - Managing evolving fish stocks. Science **318**:1247-1248.

Jorgensen, C., B. Ernande, and O. Fiksen. 2009. Size-selective fishing gear and life history evolution in the Northeast Arctic cod. Evolutionary Applications **2**:356-370.

Kuparinen, A., S. Kuikka, and J. Merila. 2009. Estimating fisheries-induced selection: traditional gear selectivity research meets fisheries-induced evolution. Evolutionary Applications **2**:234-243.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210-1226.

Law, R. 2000. Fishing, selection, and phenotypic evolution. Ices Journal of Marine Science **57**:659-668.

Malzahn, A. M., C. Clemmesen, and H. Rosenthal. 2003. Temperature effects on growth and nucleic acids in laboratory-reared larval coregonid fish. Marine Ecology-Progress Series **259**:285-293.

Millar, R. B., and R. J. Fryer. 1999. Estimating the size-selection curves of towed gears, traps, nets and hooks. Reviews in Fish Biology and Fisheries **9**:89-116.

Naceur, N., and B. Büttiker. 1999. La palée du lac de Joux: statistiques de pêche des reproducteurs; âge, croissance et fécondité. Bulletin de la Société Vaudoise des Sciences Naturelles **86**:273-296.

Nusslé, S., C. Bornand, and C. Wedekind. 2009. Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. Evolutionary Applications **2**:200-208.

Okamoto, K. W., R. Whitlock, P. Magnan, and U. Dieckmann. 2009. Mitigating fisheries-induced evolution in lacustrine brook charr (*Salvelinus fontinalis*) in southern Quebec, Canada. Evolutionary Applications **2**:415-437.

Prchalova, M., J. Kubecka, M. Riha, T. Mrkvicka, M. Vasek, T. Juza, M. Kratochvil, *et al.* 2009. Size selectivity of standardized multimesh gillnets in sampling coarse European species. Fisheries Research **96**:51-57.

Reis, E. G., and M. G. Pawson. 1999. Fish morphology and estimating selectivity by gillnets. Fisheries Research **39**:263-273.

Ricker, W. E. 1954. Stock and recruitment. Journal of the Fisheries Research Board of Canada **11**:559-623.

Ricker, W. E. 1981. Changes in the average size and average age of pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences **38**:1636-1656.

Rijnsdorp, A. D., and F. Storbeck. 1995. Determining the onset of sexual maturity from otoliths of individual female North Sea plaice, Pleuronectes platessa L. In *Recent developments in fish otolith research*, edited by D. H. Secor, J. M. Dean and S. E. Campana: University of South Carolina Press.

Sharpe, D. M. T., and A. P. Hendry. 2009. Life history change in commercially exploited fish stocks: an analysis of trends across studies. Evolutionary Applications **2**:260-275.

Smith, T. B., and L. Bernatchez. 2008. Evolutionary change in human-altered environments. Molecular Ecology **17**:1-8.

Stenseth, N. C., and E. S. Dunlop. 2009. Evolution: Unnatural selection. Nature **457**:803-804.

Stokes, K., and R. Law. 2000. Fishing as an evolutionary force. Marine Ecology-Progress Series **208**:307-309.

Swain, D. P., A. F. Sinclair, and J. M. Hanson. 2007. Evolutionary response to size-selective mortality in an exploited fish population. Proceedings of the Royal Society B: Biological Sciences **274**:1015-1022.

Theriault, V., D. Garant, L. Bernatchez, and J. J. Dodson. 2007. Heritability of life-history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). Journal of Evolutionary Biology **20**:2266-2277.

Thomas, G., and R. Eckmann. 2007. The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth - the Lake

Constance example revisited. Canadian Journal of Fisheries and Aquatic Sciences **64**:402-410.

Thomas, G., H. Quoss, J. Hartmann, and R. Eckmann. 2009. Human-induced changes in the reproductive traits of Lake Constance common whitefish (*Coregonus lavaretus*). Journal of Evolutionary Biology **22**:88-96.

Trippel, E. A. 1995. Age at maturity as a stress indicator in fisheries. Bioscience **45**:759-771.

Walsh, M. R., S. B. Munch, S. Chiba, and D. O. Conover. 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecology Letters **9**:142-148.

Yoneda, M., and P. J. Wright. 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Marine Ecology-Progress Series **276**:237-248.

Table 1: Observed and back-calculated average length- and weight-at-age, and the proportion of mature individuals per age class. Back-calculations are based on the relative distances of the year rings on scales

	Age						
	1 year	2 years	3 years	4 years	5 years	6 years	7 years
Average length (±SD) (mm)	123±20	231±36	315±41	366±45	387±47	401±52	422±62
Average weight (±SD) (g)	63±13	311±8	503±153	456±101	563±138	670±178	927±357
Mature	0%	22%	69%	95%	99.8%	100%	100%

Table 2: Parameter randomization for sensitivity analyses.

	Table selectivity function		Gaussian selectivity function	
	Min	Max	Min	Max
Gillnet encounter rate	30%	50%	30%	50%
Natural mortality	20%	40%	25%	45%
Heritability of juvenile growth (α_{g0})	0.05	0.35	0.05	0.35
Heritability of adult growth (α_{gt})	0.15	0.45	0.15	0.45
Initial juvenile growth (α_{g0})	120	130	120	130
Initial adult growth (αgt)	160	170	155	165

Table 3: Expected growth changes with sensitivity analyses (%)

Growth parameter	Management scenario	Gaussian	Table-Like	Total
Adult	Current management (45+50mm)	-0.128 ± 0.057	-0.095 ± 0.058	-0.112 ± 0.060
	Second management (45mm)	-0.058 ± 0.052	-0.014 ± 0.051	-0.036 ± 0.056
	Third management (40mm)	0.025 ± 0.043	0.065 ± 0.036	0.045 ± 0.044
Juvenile	Current management (45+50mm)	-0.072 ± 0.026	-0.066 ± 0.027	-0.069 ± 0.026
	Second management (45mm)	-0.049 ± 0.024	-0.036 ± 0.024	-0.042 ± 0.025
	Third management (40mm)	-0.018 ± 0.022	-0.003 ± 0.020	-0.010 ± 0.022

Figure 1: selectivity functions and size distributions

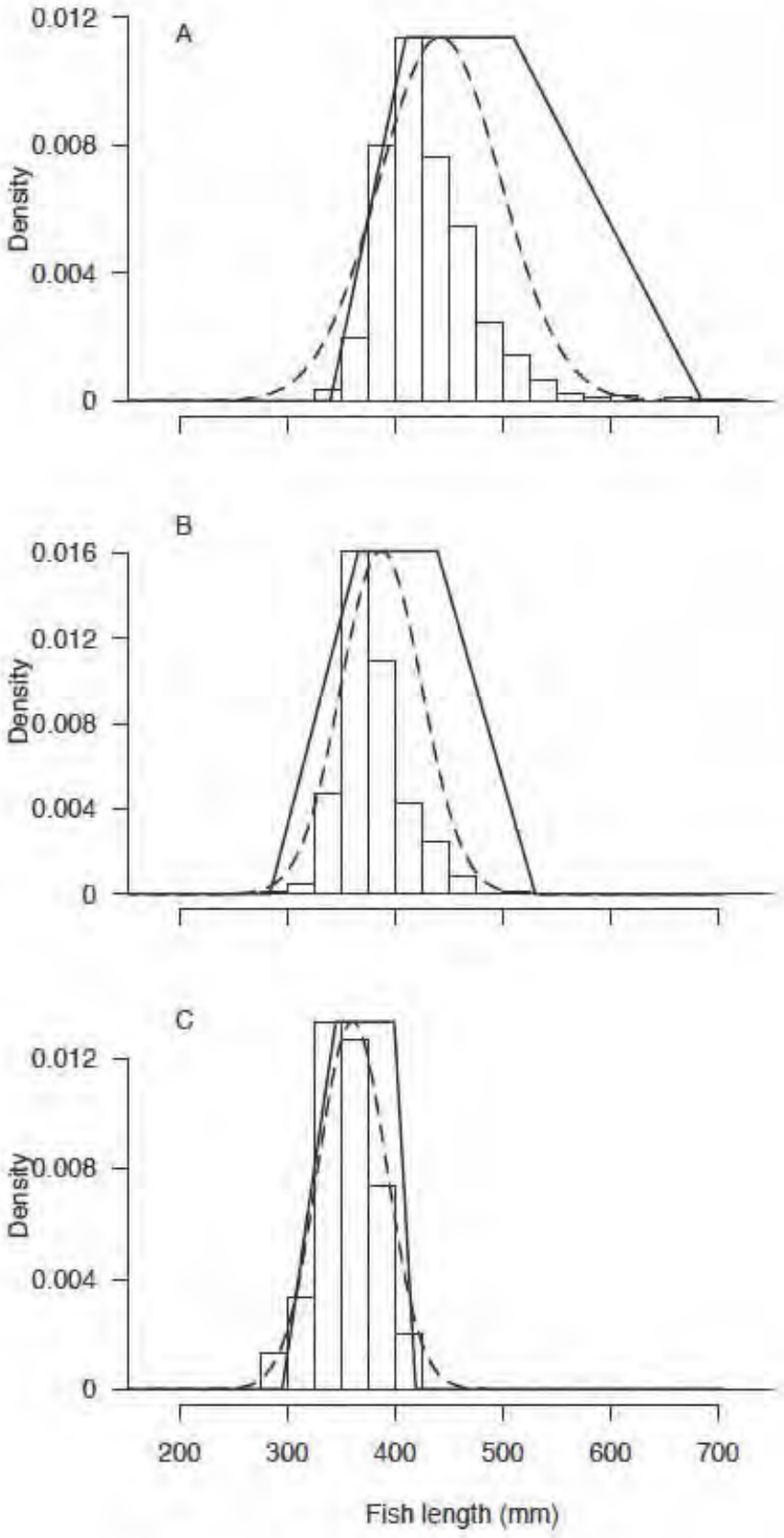


Figure 2: Age structure

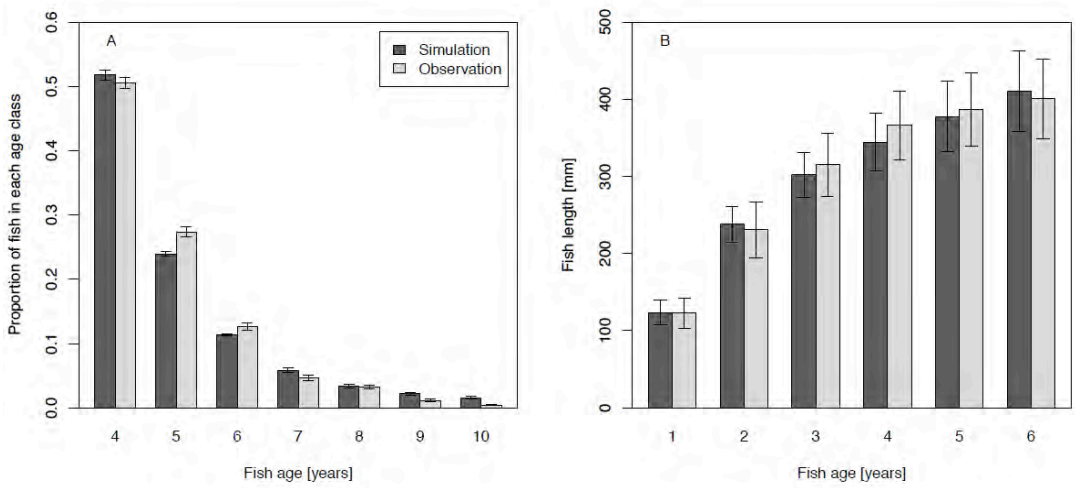


Figure 3: expected growth change

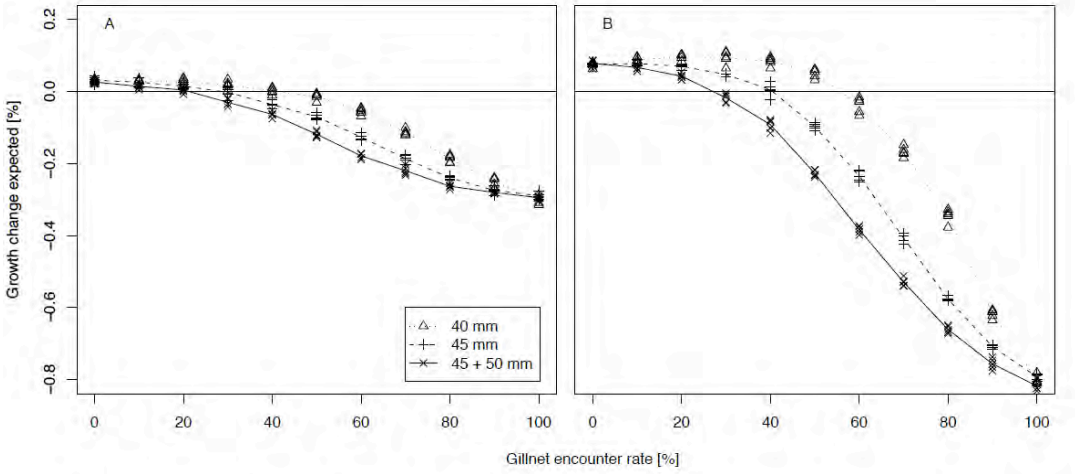


Figure 4: expected yield

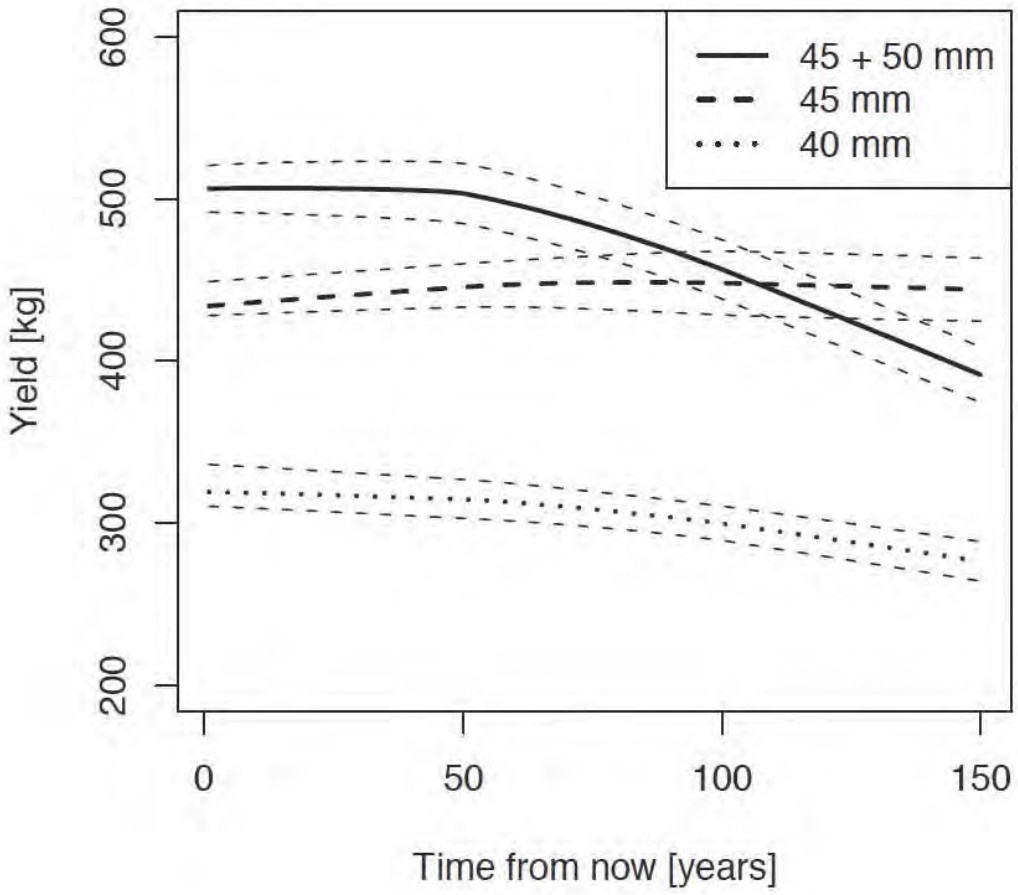


Figure 5: sensitivity analyses

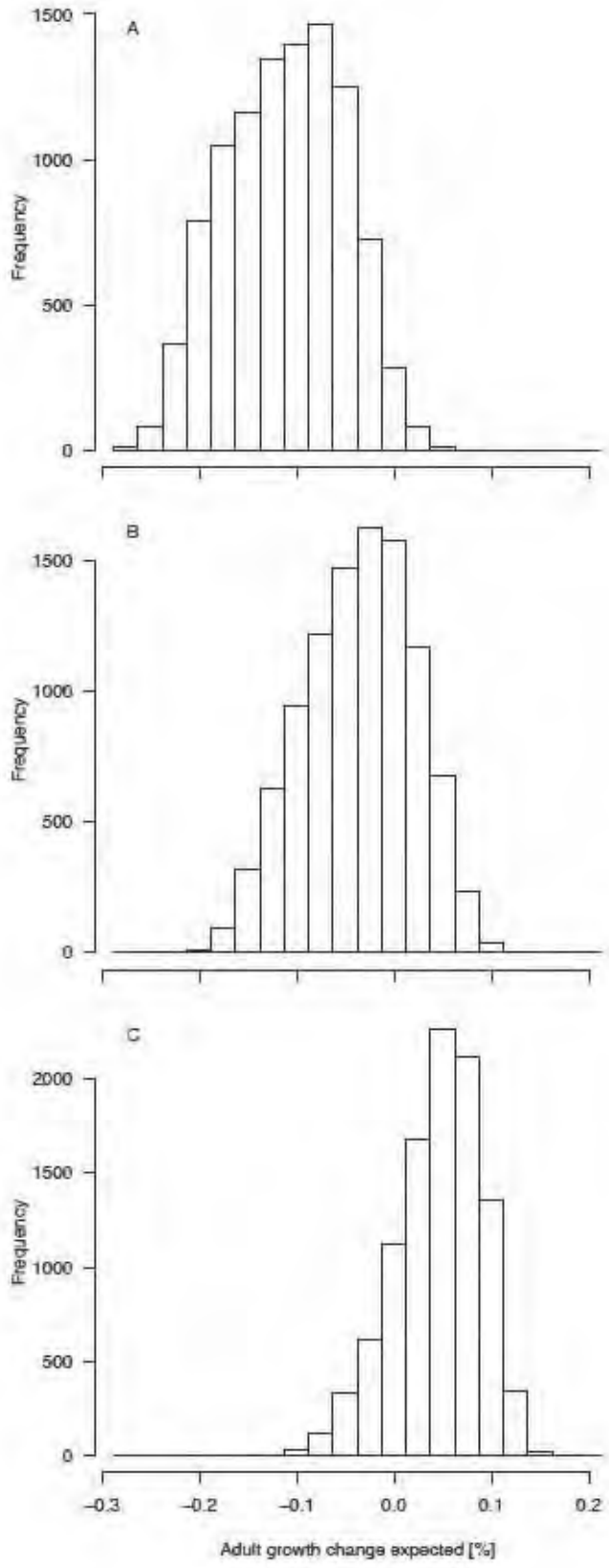


Figure legends

Figure 1: Length distribution (histogram) and modelled selectivity functions with table-like function (solid line) and Gaussian function (dashed line), for different mesh sizes (A = 50 mm, B = 45 mm, and C = 40 mm). The height of the modelled selectivity functions represents the probability for a fish to get caught by the net if encountered; the maximal value was set to one.

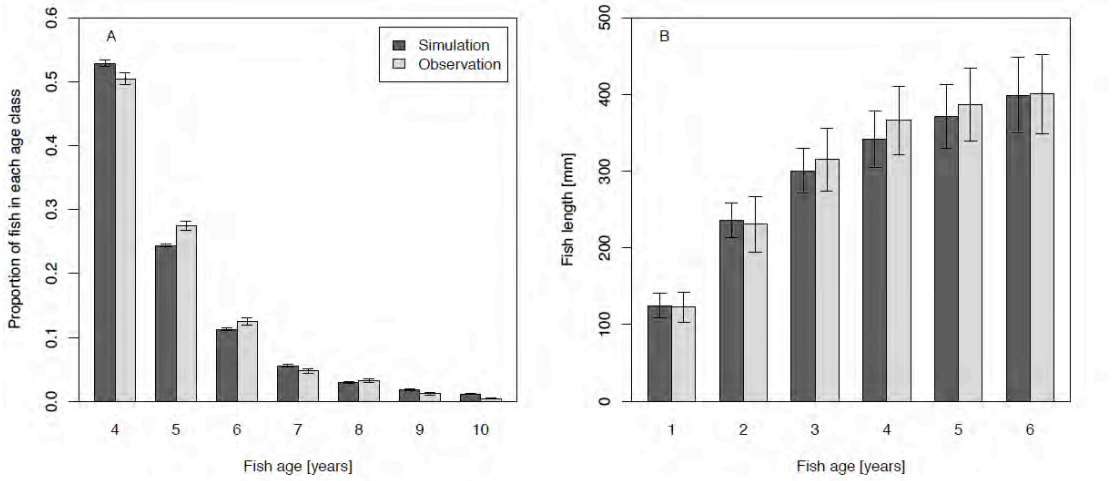
Figure 2: Convergence between modelled and real population for the table-like function (A) simulated (dark grey) and observed (light grey) age-class distribution in the population after 25 years of fishing. (B) Simulated and estimated length-at-age distribution of the same population. Model fit for the Gaussian function gave similar results, which are given as supplementary material (Supplementary Figure 1).

Figure 3: Relative growth change per year (%) for the table-like function expected after 50 years as a function of the gillnet encounter rate under three different management scenarios for juvenile growth (A) and adult growth (B): current management scenario (black curve, crosses), second management scenario (dashed curve, plus-symbols), and third management scenario (dotted curve, triangles). Each dot is calculated from a different simulation (5 per management scenario). Results for the Gaussian function are similar and are given as supplementary material (Supplementary Figure 2).

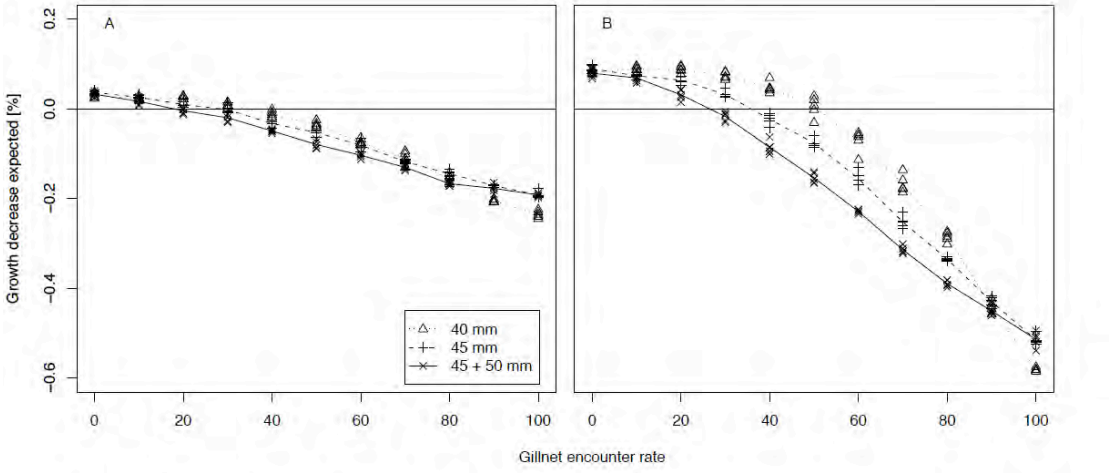
Figure 4: Expected yield of whitefish fishery in Lake Joux per year under three different management scenarios, for the table-like function: current management scenario with mesh sizes of 45 and 50 mm (solid curve), second management scenario with mesh sizes of 45 mm only (dashed curve) and third management scenario with mesh sizes of 40 mm only (dotted curve). Small dashed lines represent the 95 % confidence interval for each scenario.

Figure 5: Predicted adult growth under various assumptions. Growth change is expected to be negative with the current management scenario (A), to be around zero under the second management scenario (B), and to be positive under the third management scenario (C).

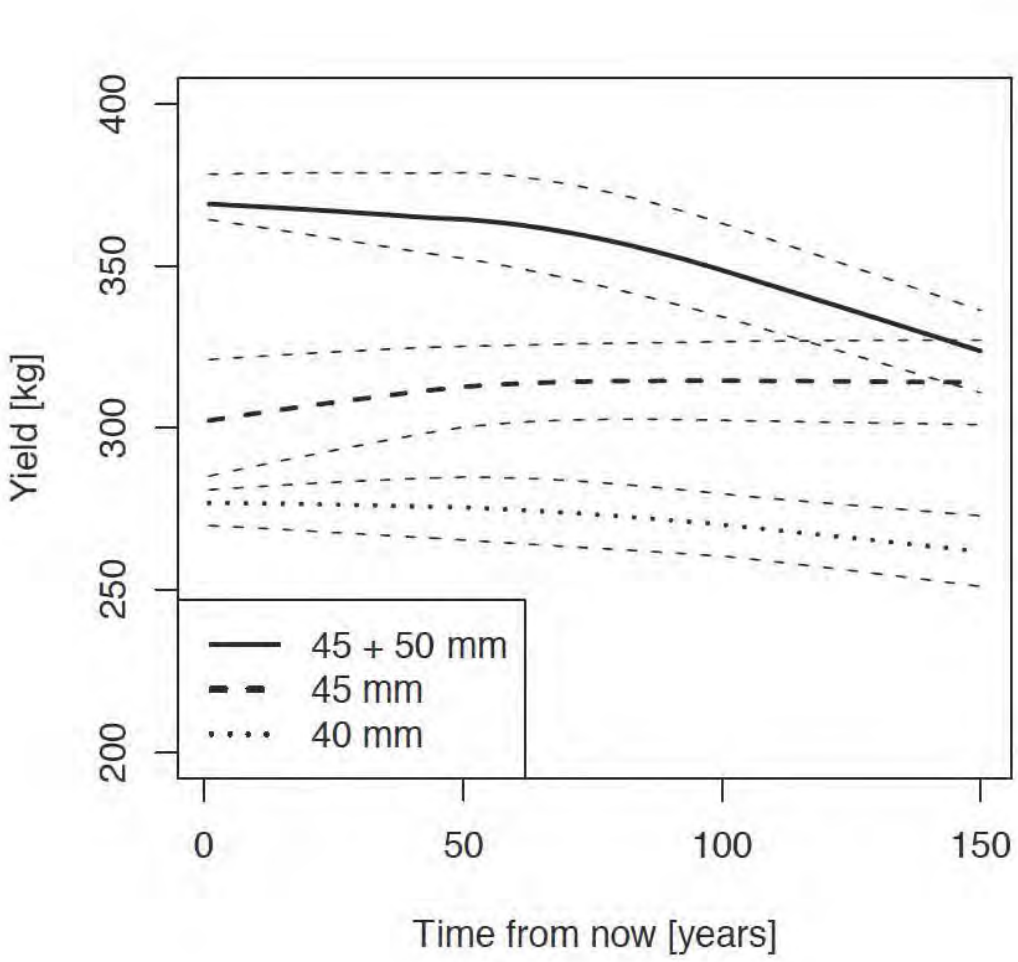
Supplementary figure 1: Age structure



Supplementary figure 2: expected growth decrease



Supplementary Figure 3: Expected yield



Supplementary Figure 1: Convergence between modelled and real population for the Gaussian function (A) simulated (dark grey) and observed (light grey) age-class distribution in the population after 25 years of fishing. (B) Simulated and estimated length-at-age distribution of the same population.

Supplementary Figure 2: Relative growth change per year (%) for the Gaussian function expected after 50 years as a function of the gillnet encounter rate under three different management scenarios for juvenile growth (A) and adult growth (B): current management scenario (black curve, crosses), second management scenario (dashed curve, plus-symbols), and third management scenario (dotted curve, triangles). Each dot is calculated from a different simulation (5 per management scenario).

Supplementary Figure 3: Expected yield of whitefish fishery in Lake Joux per year under three different management scenarios, for the Gaussian function: current management scenario with mesh sizes of 45 and 50 mm (solid curve), second management scenario with mesh sizes of 45 mm only (dashed curve) and third management scenario with mesh sizes of 40 mm only (dotted curve). Small dashed lines represent the 95 % confidence interval for each scenario.

General Discussion

We have analysed monitoring program data on five populations of four different species living in three ecologically distinct lakes, and found significant negative selection differentials on growth due to fishing pressure. In other words, since fishing on Alpine whitefish is size-selective it therefore exerts a significant selection pressure on growth. The observed selection differentials are associated with growth decreases, which are expected if growth has a genetic basis and is heritable. No heritability measures exist for growth in Alpine whitefish, but several studies on fish, in particular salmonids, have shown significant heritability with values up to 0.5 for many life-history traits (Law 2000; Garcia de Leaniz *et al.* 2007; Swain *et al.* 2007; Theriault *et al.* 2007; Carlson and Seamons 2008). Therefore, we believe that fishing has a significant effect on growth in Alpine whitefish. Moreover, with a reasonable assumption for the heritability of individual growth (h^2=0.3) fishing may contribute to a rather large proportion (about 30%) of the growth decrease observed in natural populations.

Fishing mortality may also lead to changes in reproductive strategies, where females are expected to invest more in reproduction than in self-maintenance, i.e. growth and survival, if mortality is high. Thus if females increase their allocation of resources towards reproduction any observed change in growth rate might not be solely linked to selection against fast growers (Gadgil and Bossert 1970; Heino *et al.* 2008). In our population of interest however, we did not observe any change in reproductive investment, increased fecundity, or earlier age at maturation. We can therefore exclude potential variations in the allocation of resources from growth to reproduction. Therefore the rest of the growth decrease in these populations is likely environmental, most probably linked to changes in phosphorus concentration and

hence biomass production. Most central European lakes have suffered from anthropogenetic eutrophication in the 70-80s, and this is indeed known to impact growth rates (Gerdeaux *et al.* 2006; Müller *et al.* 2007a; Müller *et al.* 2007b; Thomas and Eckmann 2007).

These results are important in the framework of fishery induced-selection: the relative importance of fishery-induced evolution versus phenotypic plasticity is indeed a key issue to be addressed (Law 2000; Law 2007; Smith and Bernatchez 2008). Separating the effects of fishing- and environmentally-induced changes on individual growth rates is usually difficult (Heino *et al*. 2008) because phenotypic plasticity is important in fish (Thorpe 1998; Crozier *et al*. 2008) and even if genetic changes can be documented over time, monitoring data alone cannot conclusively demonstrate the causal link between such genetic changes and particular changes in the environment (Hutchings & Fraser 2008). In this study, we not only provide a first estimation of the relative contributions of genetic and environmental effects on long-term phenotypic changes, but were also able to detail the relationship between these factors: within-lake comparisons indicate that the slow-growing species (Bondelle and Brienzlig) are less influenced by phosphorus changes than fast-growing species (Palée and Albock) and these slow-growing species display smaller growth decreases under equivalent selection pressure. Competition for food, a well-known evolutionary force (Landry *et al.* 2007; Vonlanthen *et al.* 2009) is a potential explanation for this pattern, as slow-growing species are more specialized to small prey (Link and Hoff 1998; Tolonen 1998; Bernatchez *et al.* 1999; Eckmann *et al.* 2002; Müller *et al.* 2007b), which are more prevalent in adverse conditions than larger prey. In addition, comparisons between lakes seem to indicate that differences between species could be influenced by environmental pressure. In Lake Brienz, where fish populations endure

the most adverse conditions, they display larger growth decreases and the highest selection differentials while in Lake Biel and Lake Joux, where the phosphorus concentration is close to the species optimum (Gerdeaux *et al.* 2006; Eckmann and Rösch 2007), selection differentials are smaller.

A decrease in growth can have deleterious consequences for populations. The average viability of populations is known to suffer from fishery-induced artificial selection (Fenberg and Roy 2008). Several specific consequences may arise from the removal of large fish, and although these issues are still debated (Carlson *et al.* 2008), a precautionary approach should be taken when managing evolving fish stocks (Francis and Shotton 1997). First, large and fast-growing individuals may be of higher genetic quality than small and slow-growing individuals (Birkeland and Dayton 2005). A systematic removal of 'high quality' adults could therefore result in an increase of the average genetic load in a population. Second, as large females usually produce larger offspring of higher viability (Trippel 1995; Walsh *et al.* 2006), the removal of larger females could impair the recruitment and consequently the long-term yield of the population. Third, as females in many species prefer to mate with large males (Wedekind *et al.* 2007; Hutchings and Rowe 2008; Rudolfsen *et al.* 2008; Jacob *et al.* 2009; Labonne *et al.* 2009) increased mortality of large fish could have an impact on sexual selection and therefore on mating behaviour. Fourth, non-random mortality could decrease the genetic diversity of the population and make it more vulnerable to environmental changes or disease(Jones *et al.* 2001).

In the first three chapters, we have shown that fishery-induced evolution impacts fish populations and this effect is likely substantial. In the fourth chapter, we demonstrate that implementing a relatively simple management scenario, i.e. a reduced catch-range of body sizes, could allow fast growers to escape fishery-induced

selection, and eventually help achieve two desirable long-term goals. Not only could it decelerate the observed decrease in growth, but it may also lead to a complete reversal of the trend, ultimately causing an increase in the average growth rate of the individuals. This management action may help to counteract the deleterious effects of fishing mentioned above and protect the genetic variability of growth in the population. A conservation focus on protecting larger (and thus older) individuals has been recommended before as larger individuals favour recruitment due to the higher survival and competitiveness of their offspring (Birkeland and Dayton 2005). Our model does not take into account the genetic diversity that may have already been lost over several decades of harvesting, and our hypothesis may hold only if sufficient diversity remains in the lake. It has indeed been shown empirically that a genetically-based reversal of fishing-induced evolution is possible if there is sufficient genetic variation in the population (Conover *et al.* 2009). The large variance in growth observed in our study population suggests that a recovery of the population may be possible.

Conclusion

Our findings highlight the tightly entangled interactions between selection and environment on the characteristics of fish populations and the importance of taking both ecological and economical factors into account when establishing management plans (Stokes & Law 2000; Ashley *et al*. 2003; Smith & Bernatchez 2008). Simple measures, such as fishing gear adjustment, could be beneficial to all parties involved. Such regulations should, however, be implemented with care. A thorough risk assessment of fishing gear selectivity, of the age structure and length-at-age distribution, and a quantification of both fishing and natural mortality is required to avoid potentially dramatic consequences. This might require several years of

monitoring, as populations are likely to fluctuate from year to year, and a strict monitoring program following the changes in regulations.

Perspectives

Future research should investigate growth decreases in other lakes. Our study only focuses on three lakes with different trophic structures and we can only estimate how fishery-induced selection is linked to environmental factors. It is necessary to monitor more lakes for more accurate estimates. For instance, several lakes in Switzerland and neighbouring countries are known to be oligotrophic (Lucerne, Thun, Waldenstadt and Annecy) or mesotrophic (Neuchatel, Constance, Zurich, Leman, Bourget).

Another potential limitation of our study is the lack of precise information on the heritability of growth in Alpine whitefish. Raising fish in fish farms cannot completely solve the issue because their captive environment is likely to display reduced environmental variance and therefore potentially impact the heritability estimation, but this method could gives us a first estimate of the heritability of growth. Moreover, raising whitefish for several years may help to precisely determine the relationship between fish growth and scale growth and therefore help to optimize growth back-calculations from annuli on scales.

Finally, fishery-induced selection is likely to influence several other traits. Although maturation seems to not have been impacted, adaptation to fishing gear through reduced girth has been shown in other species and could be investigated in our species. In addition, the impact of fishery-induced selection on sexual selection could be investigated, as both fisheries and females select for larger males.

Bibliography

Ashley, M. V., M. F. Wilson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation **111**:115-123.

Bernatchez, L., A. Chouinard, and G. Q. Lu. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, Coregonus sp., as a case study. Biological Journal of the Linnean Society **68**:173-194.

Birkeland, C., and P. K. Dayton. 2005. The importance in fishery management of leaving the big ones. Trends in Ecology & Evolution **20**:356-358.

Carlson, S. M., E. M. Olsen, and L. A. Vøllestad. 2008. Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. Functional Ecology **22**:663-673.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications **1**:222-238.

Conover, D. O., S. B. Munch, and S. A. Arnott. 2009. Reversal of evolutionary downsizing caused by selective harvest of large fish. Proceedings of the Royal Society of London B Biological Sciences **276**:2015-2020.

Eckmann, R., M. Becker, and M. Schmid. 2002. Estimating food consumption by a heavily fished stock of zooplanktivorous Coregonus lavaretus. Transactions of the American Fisheries Society **131**:946-955.

Eckmann, R., and R. Rösch. 2007. Lake Constance fisheries and fish ecology.

Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209-220.

Francis, R. I. C. C., and R. Shotton. 1997. "Risk" in fisheries management: a review. Canadian Journal of Fisheries and Aquatic Sciences **54**:1699-1715.

Garcia de Leaniz, C., I. A. Fleming, S. Einum, E. Verspoor, W. C. Jordan, S. Consuegra, N. Aubin-Horth, *et al.* 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews **82**:173-211.

Gerdeaux, D., O. Anneville, and D. Hefti. 2006. Fishery changes during re-oligotrophication in 11 peri-alpine Swiss and French lakes over the past 30 years. Acta Oecologica **30**:161-167.

Hilborn, R. 2006. Faith-based fisheries. Fisheries **31**:554-555.

Hutchings, J. A., and D. J. Fraser. 2008. The nature of fisheries- and farming-induced evolution. Molecular Ecology **17**:294-313.

Hutchings, J. A., and S. Rowe. 2008. Consequences of sexual selection for fisheries-induced evolution: an exploratory analysis. Evolutionary Applications **1**:129-136.

Jacob, A., G. Evanno, E. Renai, R. Sermier, and C. Wedekind. 2009. Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success in the minnow. Animal Behaviour **77**:823-829.

Jones, M. W., T. L. McParland, J. A. Hutchings, and R. G. Danzmann. 2001. Low genetic variability in lake populations of brook trout (*Salvelinus fontinalis*): A consequence of exploitation? Conservation Genetics **2**:245-256.

Jørgensen, C., K. Enberg, E. S. Dunlop, R. Arlinghaus, D. S. Boukal, K. Brander, B. Ernande, *et al.* 2007. Ecology - Managing evolving fish stocks. Science **318**:1247-1248.

Labonne, J., M. Augery, M. Parade, S. Brinkert, E. Prevost, M. Heland, and E. Beall. 2009. Female preference for male body size in brown trout, *Salmo trutta*: is big still fashionable? Animal Behaviour **77**:129-137.

Landry, L., W. F. Vincent, and L. Bernatchez. 2007. Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. Journal of Evolutionary Biology **20**:971-984.

Law, R. 2000. Fishing, selection, and phenotypic evolution. Ices Journal of Marine Science **57**:659-668.

Law, R. 2007. Fisheries-induced evolution: present status and future directions. Marine Ecology-Progress Series **335**:271-277.

Link, J., and M. H. Hoff. 1998. Relationships of Lake Herring (*Coregonus artedi*) gill raker characteristics to retention probabilities of zooplankton prey. Journal of Freshwater Ecology **13**:55-65.

Müller, B., D. Finger, M. Sturm, V. Prasuhn, T. Haltmeier, P. Bossard, C. Hoyle, *et al.* 2007a. Present and past bio-available phosphorus budget in the ultra-oligotrophic Lake Brienz. Aquatic Sciences **69**:227-239.

Müller, R., M. Breitenstein, M. M. Bia, C. Rellstab, and A. Kirchhofer. 2007b. Bottom-up control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquatic Sciences **69**:271-288.

Rudolfsen, G., R. Müller, D. Urbach, and C. Wedekind. 2008. Predicting the mating system from phenotypic correlations between life-history and sperm quality traits in the Alpine whitefish *Coregonus zugensis*. Behavioral Ecology and Sociobiology **62**:561-567.

Smith, T. B., and L. Bernatchez. 2008. Evolutionary change in human-altered environments. Molecular Ecology **17**:1-8.

Swain, D. P., A. F. Sinclair, and J. M. Hanson. 2007. Evolutionary response to size-selective mortality in an exploited fish population. Proceedings of the Royal Society of London B Biological Sciences **274**:1015-1022.

Theriault, V., D. Garant, L. Bernatchez, and J. J. Dodson. 2007. Heritability of life-history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). Journal of Evolutionary Biology **20**:2266-2277.

Thomas, G., and R. Eckmann. 2007. The influence of eutrophication and population biomass on common whitefish (*Coregonus lavaretus*) growth - the Lake Constance example revisited. Canadian Journal of Fisheries and Aquatic Sciences **64**:402-410.

Tolonen, A. 1998. Size-specific food selection and growth in benthic whitefish, *Coregonus lavaretus* (L.), in a subarctic lake. Boreal Environment Research **2**:387-399.

Trippel, E. A. 1995. Age at maturity as a stress indicator in fisheries. Bioscience **45**:759-771.

Vonlanthen, P., D. Roy, A. G. Hudson, C. R. Largiader, D. Bittner, and O. Seehausen. 2009. Divergence along a steep ecological gradient in lake whitefish (Coregonus sp.). Journal of Evolutionary Biology **22**:498-514.

Walsh, M. R., S. B. Munch, S. Chiba, and D. O. Conover. 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecology Letters **9**:142-148.

Wedekind, C., G. Rudolfsen, A. Jacob, D. Urbach, and R. Müller. 2007. The genetic consequences of hatchery-induced sperm competition in a salmonid. Biological Conservation **137**:180-188.

Acknowledgments

J’aimerais tout d’abord remercier l’Université de Lausanne et le Fond National Suisse de la Recherche Scientifique pour le financement. Ainsi que la Société Académique Vaudoise (SAV) et le Service des Forêts, de la Faune et de la Nature du canton de Vaud (SFFN) qui ont participé au financement de ma dernière année de thèse. Un grand merci également au Département d’Écologie et d’Evolution de l’Université de Lausanne pour le cadre de travail idéal.

J’aimerais ensuite remercier mon comité de thèse, le président Henrik Kaessmann, et mes deux experts, Jean-François Rubin et Nicolas Perrin. Je remercie également mon premier superviseur et directeur de thèse Claus Wedekind, pour l’opportunité offerte de rejoindre son groupe de travail, pour les connaissances qu’il ma transmises et pour la liberté accordée dans mes projets. J’aimerais ensuite remercier très chaleureusement Daniel Cherix, mon second directeur de thèse, pour son soutien et sa confiance. J’aimerais ensuite remercier tous mes collègues du groupe de biologie de la conservation avec qui j’ai partagé de très bons moments et qui ont participé à la création d’une ambiance de travail stimulante et enrichissante.

J’aimerais ensuite remercier les inspectorats de la pêche du SFFN et de l’Office de l’Agriculture et de la Nature du canton de Berne pour leurs précieuses collaborations, avec des remerciements particuliers à C. Moresi, C. Küng et F. Hoffman. Je souhaite également remercier les pêcheurs professionnels de la Vallée de Joux, J.-D. Meylan et Y. Meylan avec qui j’ai eu beaucoup de plaisir à travailler.

J’aimerais maintenant saluer tous mes amis « académiques » avec qui j’ai vogué sur la même galère : en particulier Arnaud, Leïla, Roumen, Tamas, Véro, Jonathan et Patrick mais aussi tous les autres, trop nombreux pour être mentionnés, avec qui j’ai partagé de fructueuses discussions scientifico-phylosophico-naturalistico-politico-et j’en passe. J’aimerais aussi remercier tous mes amis pour le bon temps partagé et pour m’avoir rappelé qu’il y a une vie en dehors de la recherche. Avec des remerciements particuliers aux « affreux de la Bourdo » pour les aventures partagées dans des mondes imaginaires qui ont été d’indispensables soupapes.

J’aimerais également remercier P. Mangold, S. Martin, F. Fasel, D. Gyggax, N. Golay, N. Perrin et M. Gilbert pour leur efficacité, leur soutien, leurs conseils et leur humanité dans la tempête.

Je tiens aussi à remercier toutes ces personnes qui ont été des sources d’inspirations : Jacques-Yves Cousteau pour les rêves de requins de ma jeunesse, Olivier Krähenbühl pour les histoires africaines and finalement: Pierre Hainard, Peter Vogel, Nicolas Perrin, Laurent Keller, Daniel Cherix et Jacques Hausser qui ont illuminé mon parcours académique.

J’aimerais finalement remercier toute ma famille pour leur support, leur amour et tout ce qui ne tiendrait pas sur une page. Mes parents, beaux-parents anciens et nouveaux, frères et sœurs et bien entendu mon fils Vadim et ma fille Anya qui me donnent l’énergie de me dépasser.

Finalement mes remerciements vont à ma femme Semira, pour son amour et sa confiance, sans qui rien n’aurait été possible et à qui je dédie ce travail. Merci pour ton indéfectible soutien.

Sébastien Nusslé

Avenue Jolimont 13
1005 Lausanne

Phone: +41 21 / 311 31 08
Mobile: +41 76 / 364 63 69
Email: snussle@gmail.com
Married with children (Vadim 4 years old, Anya 2 year old)

Skills

Trained researcher: PhD in evolutionary ecology, researcher since 2000. Scientific consultant for state fishery department and medical industry.

Biostatistics expert: assistant and lecturer in statistics, mixed-model methodology, programming in R language, Bayesian statistics, consulting for industry, …

Communication skills: excellent knowledge of public education (from primary to university), martial arts instructor, statistics teacher for medical industry, chief scout (1991-1996).

Modeling skills: individual-based modeling, matrix population model, population dynamics, …

Computer skills: Mac OSX, Windows, Office, Statistical softwares, moodle, basic knowledge of ArcGIS, notions of programming in Pascal and Java, notions of HTML.

Biodiversity: responsible of the zoological part of a biodiversity study in Mali (2003) financed by WWF, see www.hombori.org, trainee on Wood Ants in Swiss National Park (2001), good experience in trapping micro-mammals, founder member of a Naturalist Club "Les Blaireaux" (2002), bird, bats, mammals and amphibians watcher.

Language spoken: French (maternal), English (fluent, written and spoken), German (scholar knowledge), Spanish (notions).

Education

2012	PhD in Ecology and Evolution, Lausanne University, Switzerland
2011	Master of Advanced Studies, HEPL, Lausanne, Switzerland
2005	Diploma of Statistics, Neuchâtel University, Switzerland
2002	Master and Bachelor of Biology, Lausanne University, Ecology Institute, Switzerland

Academic and Research experience

2012 -	Assistant in Conservation Biology, University of Bern, Switzerland: statistics expert
2011 -	Researcher for "*Maison de la Rivière*", Foundation for freshwater management, Switzerland: Global warming impact on Swiss rivers and Whitefish management (*Coregonus palaea*) in Lake Joux
2009 - 2010	Statistics consulting for medical industry and medical research, Switzerland: sperm characteristics and fecundity in humans, smoking cessation and metabolism, organ transplantation and kidney function.
2006 -	Scientific consulting for state nature department, SFFN, Vaud, Switzerland: whitefish fishing management
2006 - 2010	PhD at Lausanne University, Switzerland: fishery-induced selection and conservation measures of alpine whitefish
2004 - 2010	Research assistant, Lausanne University: sperm competition and sexual behavior of shrews and Biotic costs needed for immune system mobilization by *Microtus arvalis* and population genetics of bat acarian parasites.
2004	Master thesis: spatial autocorrelation modeling in the framework of habitat prediction models
2003 & 2005	Responsible of the zoological part of a biodiversity study on Mount Hombori (Mali)
2002	Master thesis: immune system mobilization by *Microtus arvalis*
2001 - 2010	Teaching assistant, Lausanne University: statistics, scientific writing, conservation biology, and zoology.

Fundraising

Maison de la rivière: 20'000 CHF for a project on fishing induced evolution on Minnows.

Société Académique Vaudoise: 8'000 CHF for extending PhD salary.

Vaud state fishery department: ~30'000 CHF for different projects on whitefish management.

Supervisions

2006	Christophe Bornand (civil servant) - research assistant.
2007	Sabrina Guduff (trainee) – research assistant.
2008	Sophie Cotting, Tristan Deléglise, Eric Gehring, Matteo Tanadini & Lucas Villard (master students) - ABO blood group mate preferences in human.
2008	Carole Pusterla (master student) - Supportive breeding efficiency of Alpine whitefish at different trophic states of the lake.
2008	Mateo Tanadini & Eric Gehring (master students) - Is sexual selection a potential factor explaining ABO blood 1 group frequencies and distribution in humans?
2008	Mathias Beysard & Sébastien Biollay (master students) - Density dependence and fisheries impacts on cycling dynamics of alpine whitefish.
2008	Tamas Szekely (trainee) – research assistant
2009	Sarah Burgy & Ester Luzio (master students) - Is supportive breeding still necessary in oligotrophic lake?
2009	Patrice Pfäuti (master student) - The effects of fishing on sexual display.
2012	Sascha Wellig (master student) – Effect of wind turbines on bat abundance.

Teaching Experience

2012 -	Science Teacher in Nyon Highschool
2012 -	Taichi (martial art) instructor
2011 - 2012	Science Teacher in Gland public school
2010 - 2011	HEP Trainee in Nyon Highschool
2009 - 2011	Taichi (martial art) instructor
2004 - 2010	Teaching and research assistant, Lausanne University (statistics, scientific writing, conservation biology, zoology)
2001 - 2004	Science teacher in public schools
2001 - 2002	Teaching assistant (zoology), Lausanne University

Other Experience

2009 -	Associate Judge
1997 - 1999	Swimming guardian at the swimming pool of Gland (Switzerland)
1996	Landscaper help in New-Jersey (USA)
1994 - 2002	Cashier and storekeeper, Denner SA (Switzerland)

Hobbies

Martial Arts (Black belt, 1 Dan, Taichi instructor), hiking, scuba diving, snowboarding, comics, travelling

Publications (peer reviewed)

Arlettaz R., Maurer M., Mosimann P., Nusslé S., Abadi F. & Schaub M. (2011). New vineyard cultivation practices create patchy ground vegetation, favouring Woodlarks. Journal of Ornithology.

Giorgi M.S., Arlettaz R., Guillaume F., Nusslé S., Ossola C., Vogel P., Christe P. (2004). Causal mechanisms underlying host specificity in bat ectoparasites. Oecologia 138(4), 648-654.

Jacob A., Nusslé S., Britschgi A., Evanno G., Muller R. & Wedekind C. (2007). Male dominance linked to size and age, but not to 'good genes' in brown trout (Salmo trutta). Bmc Evol Biol, 7.

Laugen A. T. *et al*. (*in press*). Evolutionary impact assessment: Accounting for evolutionary consequences of fishing in an ecosystem approach to fisheries management. Fish and Fisheries.

Nusslé S., Bréchon A. & Wedekind C. (2010). Change in individual growth rate and its link to gillnet fishing in two sympatric whitefish species. Evolutionary Ecology.

Sébastien Nusslé – Avenue Jolimont 13 – 1005 Lausanne – +41 76 / 364 6369 – snussle@gmail.com

Nusslé S., Bornand C. & Wedekind C. (2009). Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. Evolutionary Applications, 2(2), 200-208.

Parapanov R., Nusslé S., Crausaz M., Senn A., Hausser J. & Vogel P. (2008). Testis size, sperm characteristics and testosterone concentrations in four species of shrews (Mammalia, Soricidae). Anim Reprod Sci, 114(1-3), 269-278.

Parapanov R., Nusslé S., Hausser J. & Vogel P. (2008). Histological description of seminiferous epithelium and cycle length of spermatogenesis in the water shrew Neomys fodiens (Mammalia : Soricidae). Anim Reprod Sci, 107, 148-160.

Parapanov R., Nusslé S., Hausser J. & Vogel P. (2008). Relationships of basal metabolic rate, relative testis size and cycle length of spermatogenesis in shrews (Mammalia, Soricidae). Reprod Fert Develop, 20, 431-439.

Parapanov R., Nusslé S. & Vogel P. (2007). Cycle length of spermatogenesis in shrews (Mammalia : Soricidae) with high and low metabolic rates and different mating systems. Biology of Reproduction, 76, 833-840.

Wedekind C., Jacob A., Evanno G., Nusslé S. & Muller R. (2008). Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers. Proceedings of the Royal Society B-Biological Sciences, 275, 1737-1744.

Publications (*in prep*)

Arlettaz,R. Genoud M., Nusslé S. et al. (*in prep*). Disturbance of wildlife by outdoor winter recreation: funnel-shaped allostatic stress response and altered activity-energy budgets in Alpine Black grouse

Gonseth S., Bize R., Nusslé S., Pralong F., Willi C. & Cornuz J. (*submitted*). Leptin pattern during smoking cessation. Addiction.

Heino M. et al. (*in prep*). Can fisheries-induced evolution shift reference points for fisheries management?

Nusslé S. et al. (*in prep*). Investigating the link between fishery-induced evolution, environmental changes and species-specific trophic niche.

Nusslé S., Presi P., Kawecki T. & Wedekind C. (*in prep*). Towards reconciling fishery and fishery-induced evolution: predicting the effects of changed mesh size regulations.

Parapanov, R., Mendiola, J., Nusslé S., Crausaz, M.Vargas J., Stettler E. Wisard, M., Germond, M., Senn, A. (submitted). Volume and function of testes in relation to body height in young Swiss men. International Journal of Andrology.

Parapanov R., Salamin N., Nusslé S., Hausser J. & Vogel P. (submitted). The relative influence of body mass, metabolic rate and sperm competition on the spermatogenic cycle length in mammals.

Publications (other)

Jacob A., Nusslé S, von Siebenthal B. and Wedekind C. (2009). Stress-induced change in the quantitative genetics of hatching timing in brown trout. Alain Jacob PhD thesis chapter 7, University of Lausanne, Lausanne, Switzerland.

Nusslé S. (2009). Palées du Lac de Joux : Synthèse 2006-2008. Internal report. Service de la Faune, de la Forêt et de la Nature Saint-Suplice, Suisse.

Nusslé S. (2011). Palées du Lac de Joux : Monitoring 2009-2010. Internal report. Service de la Faune, de la Forêt et de la Nature Saint-Suplice, Suisse.

Nusslé S. (2012). Palées du Lac de Joux : Monitoring 2010-2011. Internal report. Service de la Faune, de la Forêt et de la Nature Saint-Suplice, Suisse.

Nusslé S. & Bornand C. (2007). Compte rendu de l'Evolution de la population de palées (Coregonus palaea) du lac de Joux. Internal report. Service de la Faune, de la Forêt et de la Nature Saint-Suplice, Suisse.

Nusslé S., Guduff S. & Bornand C. (2008). Evolution de la population de palées (Coregonus palaea) du lac de Joux entre 1980 et 2007 et réfléxions sur sa gestion. Internal report. Service de la Faune, de la Forêt et de la Nature, Suisse.

von Siebenthal B., Jacob A., Nusslé S. and Wedekind C. (2009). Genetic and pathogen-linked effects on the timing of hatching in a salmonid. Beat von Siebenthal PhD thesis chapter 3, University of Lausanne, Lausanne, Switzerland.

Public presentations

June 2012	L'Abbaye, Switzerland. Consultative assembly for fishing in Lake Joux: Palée du lac de Joux, Synthèse 2006 – 2011 (oral presentation).
June 2011	L'Abbaye, Switzerland. Consultative assembly for fishing in Lake Joux: Palée du lac de Joux, Synthèse 2006 – 2010 (oral presentation).
June 2010	L'Abbaye, Switzerland. Consultative assembly for fishing in Lake Joux: Palée du lac de Joux, Synthèse 2006 – 2009 (oral presentation).
Feb. 2010	Neuchâtel, Switzerland. Biology10 : Past and future fishery-induced evolution on growth in an Alpine Whitefish (oral presentation)
Nov. 2009	Berlin, Germany. International Conference on Evolutionary Ecology of Fishes : Fishery-induced evolution on growth and management options (oral presentation)
Sept. 2009	Prague, Tcheque Republic. European Congress of Conservation Biology : Fishery-induced rapid evolution and fi shing-gear adjustment in population management (oral presentation)
June 2009	Le Sentier, Switzerland. Consultative assembly for fishing in Lake Joux: Rapport de synthèse des données récoltées entre 2006 et 2008 et suivi des propositions de gestion (oral presentation).
Feb. 2009	Bern, Switzerland. Biology09: Fishery-induced rapid evolution and fishing gear adjustment in population management (poster).
June 2008	Le Sentier, Switzerland. Consultative assembly for fishing in Lake Joux: Compte rendu de l'évolution de la population de palées du lac de Joux (oral presentation).
April 2008	Konstanz, Germany: University of Konstanz, invited speaker: Fishery-induced selection on Alpine whitefish: quantifying genetic and environmental effects on growth rate (oral presentation).
Feb. 2008	Lausanne, Switzerland. Biology08: Fishery-induced selection on Alpine whitefish: quantifying genetic and environmental effects on growth rate (oral presentation).
Jan. 2008	Lausanne, Switzerland. Hotspots Training course: Evolutionary Processes, Molecular Tools and Bioinformatics. GLM and LMM models in R (invited lecturer).
Jan. 2008	Kastanienbaum, Switzerland. Swiss Federal Institute of Aquatic Science and Technology (EAWAG): Fishery-induced selection on Alpine whitefish: quantifying genetic and environmental effects on growth rate (oral presentation).
Jan. 2008	Copenhagen, Denmark. Study-Group on Fishery-Induced Adaptive Change: Fishery-induced selection on Alpine whitefish: quantifying genetic and environmental effects on growth rate (oral presentation).
Nov. 2007	Lausanne, Switzerland. University of Lausanne, Department of Ecology and Evolution, Internal Seminar: Fishery-induced selection on Alpine whitefish: quantifying genetic and environmental effects on growth rate (oral presentation).
Aug. 2007	Uppsala, Sweden. 11th Congress of the European Society for Evolutionary Biology: Fishery-induced decrease in individual growth rates: disentangling genetic from environmental effects (poster).
June 2007	Le Sentier, Switzerland. Consultative assembly for fishing in Lake Joux: Compte rendu de l'évolution de la population de palées du lac de Joux (oral presentation).
March 2007	Lausanne, Switzerland. University of Lausanne D.Day: Fishery-induced decrease in individual growth rates: disentangling genetic from environmental effects (poster).
Sept. 2006	St. Andrews, Scotland. 12th Annual European Meeting of PhD students in Evolutionary Biology: Phenotypic changes in alpine coregonids revealed from monitoring surveys covering up to 60 years (oral presentation).
Aug. 2006	Eger, Hungary. 1st European Congress of Conservation Biology: Phenotypic changes in Alpine coregonids revealed from monitoring surveys that cover up to 60 years (poster).

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole doctorale

Procès-verbal

Lausanne, le 24 mai 2012

Monsieur
Sébastien Nusslé
Avenue Jolimont 13
1005 Lausanne

N°immat.: 95407748
né le 20.04.1976

Doctorat ès sciences de la vie

	Statut / Note	Crédits	Moyenne / Modalité	Semestre / Session
Procédure de thèse				
Rapport annuel 1	Réussi		V	01/2008
Crédits ECTS : exigences de première année	Réussi		V	08/2009
Exigences de fin de première année	Réussi		V	08/2009
Rapport annuel 2	Réussi		V	06/2009
Rapport annuel 3	Réussi		V	06/2010
Evaluation intermédiaire	Réussi		V	06/2009
Crédits ECTS : exigences du programme doctoral	Réussi		V	01/2010
Tutorials		**1.00**		
Statistiques appliquées aux projets de recherche	Réussi	1.00	V	06/2009
Equivalent à un tutorial		**1.00**		
Groupe de discussion en Ecologie et Evolution: session 1 Evolutionary Conservation Biology *Université de Lausanne*	*Attesté*			2007A
Groupe de discussion en Ecologie et Evolution: session 1	Réussi	1.00	V	01/2008
Cours de 3e cycle		**3.00**		
3e Cycle Romand en Sciences Biologiques Workshop "Managing adaptive genetic variation in conservation biology", La Fouly (CH), 03-06.09.2008 *Suisse*	*Attesté*	1.50		2008A
Cours de 3e cycle New insights into mixed model methodology with applications to genomics and biostatistics, La-Londes-les-Maures (FR), 21-25.05.2007 *France (autres institutions)*	*Attesté*	1.50		2007E
Séries de séminaires		**1.00**		
Ecologie et évolution	*Attesté*			2007A
Série de séminaires	Réussi	1.00	V	01/2008

Cette décision est immédiatement exécutoire.
Nombre de crédits ECTS requis pour le Doctorat ès sciences de la vie : 12
Note maximale: 6; Note suffisante: 4; Note minimale: 0
V = Validation

Attestation sans signature.
Veuillez conserver ce document.

Adresse pour correspondance : Université de Lausanne, Faculté de biologie et de médecine, Ecole doctorale, Quartier UNIL-Sorge, Bâtiment Biophore, CH - 1015 Lausanne

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole doctorale

Procès-verbal

Lausanne, le 24 mai 2012

Monsieur
Sébastien Nusslé
Avenue Jolimont 13
1005 Lausanne

N°immat.: 95407748
né le 20.04.1976

Doctorat ès sciences de la vie

	Statut / Note	Crédits	Moyenne / Modalité	Semestre / Session
Congrès		**6.00**		
Congrès 1st European congress of conservation biology - ECCB, Eger (HU), 22-27.08.2006 *Hongrie (autres institutions)*	*Attesté*	1.00		2006H
Congrès 11th Congress of the European Society for Evolutionary Biology - ESEB XI, Uppsala (SE), 20-25.08.2007 *Suède (autres institutions)*	*Attesté*	1.00		2007A
Congrès Biology 2008, Lausanne (CH), 6-7.02.2008 *Suisse*	*Attesté*	1.00		2008P
Congrès Biology 2009, Berne (CH), 12-13.02.2009 *Suisse*	*Attesté*	1.00		2009P
Congrès 2nd European Congress of Conservation Biology - ECCB, Prague (CZ), 01-05.09.2009 *Rép. Tchèque (autres villes)*	*Attesté*	1.00		2009A
Congrès International conference on evolutionary ecology of fishes, Erkner (DE), 23-25.11.2009 *Allemagne (autres institutions)*	*Attesté*	1.00		2009A
Autres événements		**0.50**		
D. Day Lausanne (CH), 14.02.2008 *Université de Lausanne*	*Attesté*	0.50		2008P

Cette décision est immédiatement exécutoire.
Nombre de crédits ECTS requis pour le Doctorat ès sciences de la vie : 12
Note maximale: 6; Note suffisante: 4; Note minimale: 0
V = Validation

Attestation sans signature.
Veuillez conserver ce document.

Adresse pour correspondance : Université de Lausanne, Faculté de biologie et de médecine, Ecole doctorale, Quartier UNIL-Sorge, Bâtiment Biophore, CH - 1015 Lausanne

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole doctorale

Procès-verbal

Lausanne, le 24 mai 2012

Monsieur
Sébastien Nusslé
Avenue Jolimont 13
1005 Lausanne

N°immat.: 95407748
né le 20.04.1976

Doctorat ès sciences de la vie

	Statut / Note	Crédits	Moyenne / Modalité	Semestre / Session
Activité de formation doctorale		**4.00**		
Activité Evolutionary Conservation Biology, Discussion Group, Lausanne (CH), 20.03-12.06.2006 *Université de Lausanne*	*Attesté*	1.00		2006H
Activité Academic writing (Niveau B2/C1), Lausanne (CH), nov.05-juin 06 *Université de Lausanne*	*Attesté*	2.00		2006H
Activité 12th annual meeting of the PhD Students in evolutionary biology, St Andrews (GB), 04-09.09.2006 *Grande-Bretagne (autres institutions)*	*Attesté*	1.00		2006H
Autres programmes doctoraux		**3.50**		
Inter-University Doctoral Program in Ecology and Evolution Course: How to sell science to good journals, Lausanne (CH), 5-11-26.11.2007 *Université de Lausanne*	*Attesté*	1.00		2007A
Inter-University Doctoral Program in Ecology and Evolution Course : Foundations of the theory of speciation, Lausanne (CH), 05-06.11.2008 *Université de Lausanne*	*Attesté*	0.50		2008A
Inter-University Doctoral Program in Ecology and Evolution Course : Introduction to field animal experimentation, Lausanne (CH), 23-25.03.2009 et 08-12.06.2009 *Université de Lausanne*	*Attesté*	1.00		2009P
Inter-University Doctoral Program in Ecology and Evolution Course : Mixed models in statistics, Lausanne (CH), 01-03.07.2009 *Université de Lausanne*	*Attesté*	1.00		2009P

Résultat: **Intermédiaire**
Crédits ECTS: **20.00**

Cette décision est immédiatement exécutoire.
Nombre de crédits ECTS requis pour le Doctorat ès sciences de la vie : 12
Note maximale: 6; Note suffisante: 4; Note minimale: 0
V = Validation

Attestation sans signature.
Veuillez conserver ce document.

Adresse pour correspondance : Université de Lausanne, Faculté de biologie et de médecine, Ecole doctorale, Quartier UNIL-Sorge, Bâtiment Biophore, CH - 1015 Lausanne

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole doctorale

Procès-verbal

Lausanne, le 24 mai 2012

Monsieur
Sébastien Nusslé
Avenue Jolimont 13
1005 Lausanne

N°immat.: 95407748
né le 20.04.1976

Doctorat ès sciences de la vie

Cette décision est immédiatement exécutoire.
Nombre de crédits ECTS requis pour le Doctorat ès sciences de la vie : 12
Note maximale: 6; Note suffisante: 4; Note minimale: 0
V = Validation

Attestation sans signature.
Veuillez conserver ce document.

Adresse pour correspondance : Université de Lausanne, Faculté de biologie et de médecine, Ecole doctorale, Quartier UNIL-Sorge, Bâtiment Biophore, CH - 1015 Lausanne

Appendix I - Publications in the context of fish evolution

Male dominance linked to size and age, but not to 'good genes' in brown trout (Salmo trutta)

Alain Jacob, Sébastien Nusslé, Adrian Britschgi, Guillaume Evanno, Rudolf Müller, Claus Wedekind

Published in BMC Evolutionary Biology

Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers

Claus Wedekind, Alain Jacob, Guillaume Evanno, Sébastien Nussle and Rudolf Müller

Published in Proceedings of the Royal Society of London B

Genetic and pathogen-linked effects on the timing of hatching in a salmonid

Beat A. von Siebenthal, Alain Jacob, Sébastien Nusslé and Claus Wedekind

Thesis Chapter in B. von Siebenthal PhD thesis & in Alain Jacob thesis

---

Stress-induced change in the quantitative genetics of hatching timing in brown trout

Alain Jacob, Sébastien Nusslé Beat A. von Siebenthal and Claus Wedekind

Thesis Chapter in Alain Jacob thesis & B. von Siebenthal PhD thesis

---

BMC Evolutionary Biology

Research article

Male dominance linked to size and age, but not to 'good genes' in brown trout (*Salmo trutta*)

Alain Jacob*[1,2], Sébastien Nusslé[1], Adrian Britschgi[2], Guillaume Evanno[1], Rudolf Müller[3] and Claus Wedekind[1,2,3]

Address: [1]Department of Ecology and Evolution, University of Lausanne, Biophore, 1015 Lausanne, Switzerland, [2]Division of Conservation Biology, University of Bern, Erlachstrasse 9a, 3012 Bern, Switzerland and [3]Eawag: Swiss Federal Institute of Aquatic Science and Technology, Seestrasse 79, 6047 Kastanienbaum, Switzerland

Email: Alain Jacob* - alain.jacob@unil.ch; Sébastien Nusslé - sebastien.nussle@unil.ch; Adrian Britschgi - adrian.britschgi@dkf.unibe.ch; Guillaume Evanno - guillaume.evanno@unil.ch; Rudolf Müller - rudolf.mueller@eawag.ch; Claus Wedekind - claus.wedekind@unil.ch

* Corresponding author

Published: 1 November 2007

BMC Evolutionary Biology 2007, **7**:207 doi:10.1186/1471-2148-7-207

Received: 19 July 2007
Accepted: 1 November 2007

This article is available from: http://www.biomedcentral.com/1471-2148/7/207

Abstract

Background: Males that are successful in intra-sexual competition are often assumed to be of superior quality. In the mating system of most salmonid species, intensive dominance fights are common and the winners monopolise most mates and sire most offspring. We drew a random sample of mature male brown trout (*Salmo trutta*) from two wild populations and determined their dominance hierarchy or traits linked to dominance. The fish were then stripped and their sperm was used for *in vitro* fertilisations in two full-factorial breeding designs. We recorded embryo viability until hatching in both experiments, and juvenile survival during 20 months after release into a natural streamlet in the second experiment. Since offspring of brown trout get only genes from their fathers, we used offspring survival as a quality measure to test (i) whether males differ in their genetic quality, and if so, (ii) whether dominance or traits linked to dominance reveal 'good genes'.

Results: We found significant additive genetic variance on embryo survival, i.e. males differed in their genetic quality. Older, heavier and larger males were more successful in intra-sexual selection. However, neither dominance nor dominance indicators like body length, weight or age were significantly linked to genetic quality measured as embryo or juvenile survival.

Conclusion: We found no evidence that females can improve their offspring's genetic viability by mating with large and dominant males. If there still were advantages of mating with dominant males, they may be linked to non-genetic benefits or to genetic advantages that are context dependent and therefore possibly not revealed under our experimental conditions – even if we found significant additive genetic variation for embryo viability under such conditions.

Background

In mating systems with elaborate male-male competition, the winners usually get most mates and sire most of the offspring [1-10]. Such a skewed male mating success may either be explained by physically limited access of subdominant males to females and/or by female preference for dominant males [11-13]. Females may prefer more dominant and more attractive males because they provide more resources, better parental care [14,15] or better genes for the common offspring [2,16-18]. The

latter hypothesis corresponds to the so-called 'good-genes' hypotheses of sexual selection, i.e. variation in genetic quality is then predicted to be linked to male characteristics that influence female mate choice. The problem of how such genetic variation can be maintained under sexual selection is known as the "lek paradox" [19], and a number of possible solutions for this paradox have been offered (reviewed in [20,21]). Although it is still not fully clear how the genetic variation is maintained, there is much evidence in various species that females can gain genetic advantages by preferring males with well-developed attractiveness traits [22]. Whether females gain genetic benefits by mating with dominant males is less clear.

Experimental tests of the 'good-genes' hypotheses of sexual selection usually suffer from at least one of two problems: First, the predicted genetic effects could be confounded with non-genetic effects. This is especially so in species with some form of parental care. Males with more elaborate secondary sexual characters could, for example provide good genes and much paternal care [23,24]. Second, females sometimes adjust their investment in the offspring (e.g. yolk quality in egg) according to their perception of male attractiveness [25,26]. As a consequence of such differential allocation, 'good genes' effects can be confounded with maternal effects. However, some recent *in vitro* fertilization experiments could control for these potential confounding factors. They demonstrate that offspring viability can indeed have a genetic basis that is revealed by potential attractiveness traits [27,28]. In salmonids, not much is known about female preference for attractiveness traits, but males usually fight intensely for access to spawning territories or to females, i.e. intra-sexual selection is often very important [1,8,29-34]. Females seem to generally prefer spawning with dominant males [8,35,36]. Here we test whether male characteristics that are important in intra-sexual selection are also linked to genetic quality.

'Genetic quality' is, in the context of sexual selection, an umbrella term that includes additive ('good genes') and non-additive genetic effects ('compatible genes') on offspring survival [16]. If male dominance is linked to genetic quality and also positively to breeding success, we predict dominance to be linked to the additive genetic variance in fitness, i.e. to variation in 'good genes', since only additive genetic effects can lead to an universally valid order of mate quality while with non-additive genetic effects the order of mate quality would differ for different females [18]. Because embryogenesis is a crucial life-history stage with usually high mortalities [37,38], and male brown trout provide only genes to their offspring, we used embryo survival as a measure of genetic quality, and we used full-factorial breeding designs to separate and compare additive and non-additive genetic effects on embryo survival.

In a first experiment we caught brown trouts (*Salmo trutta forma fario*, Salmonidae), shortly before spawning season and released them into an artificial channel to study intra-sexual selection. We used the outcomes of all male fights to construct a dominance hierarchy and to test whether there are male characteristics that are linked to dominance. We stripped the fish and used their gametes in a 10 males × 8 females full-factorial breeding design (North Carolina II design [39]). The embryos of the resulting 80 families were raised individually under controlled conditions. We then tested whether males differed in their genetic quality, and if so, whether dominance indicators are linked to superior genetic quality. The last two questions were tested again in a second experiment where we determined and analysed embryo and juvenile survival of additional 13 brown trout males from another river. In the first experiment we found that older, heavier and larger males are more dominant in male-male interactions. In both experiments males differ in their genetic quality, but dominant males do not seem to be of superior genetic quality.

Results

First experiment

Two males were 2 years old, 5 males were 3 years old and 3 males were 4 years old (their body lengths are plotted in Figure 1). Male age, body weight, and body length were all strongly correlated to each other (r always ≥ 0.93, n = 10, p always < 0.0001). Larger males were on average more dominant (Figure 1; with David's score (DS): Spearman's rank order correlation coefficient $r_s = 0.75$, $p = 0.015$; and with Clutton-Brock et al.'s index (CBI): $r_s = 0.68$, $p = 0.035$). We found analogous positive relationship between dominance and male age (DS: $r_s = 0.82$, $p = 0.006$; CBI: $r_s = 0.72$, $p = 0.02$) or male weight (DS: $r_s = 0.73$, $p = 0.02$; CBI: $r_s = 0.64$, $p = 0.05$, n always = 10).

Average embryo survival was 77.9% (± 11.3 s.d.). Offspring of different females differed in their survival as the female effect explained a significant part of the variance in offspring mortality (the model without female effect (*male model*) differed significantly in its goodness of fit from the *reference model*; Table 1). Males also differed significantly in their offspring survival (i.e. the model without the male effect (*female model*) explains significantly less variance in offspring mortality than the *reference model*; Table 1). We found no significant male × female interaction effect on embryo survival (Table 1). The *AIC*s of the different models and the differences between the *AIC*s also indicate that the *reference model* is the most parsimonious one that fits our data best (see Table 1 for details). The fixed temperature effect in the *reference model*

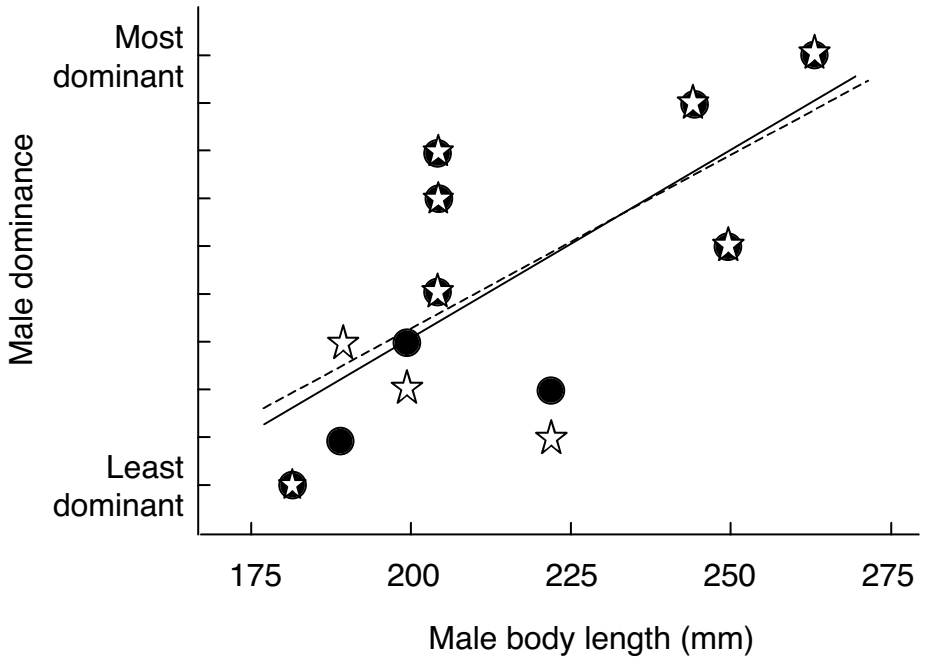


Figure 1
The effect of male body length on dominance in male-male interactions. Dominance is given as David's score (circles and non-dashed regression line) and as Clutton-Brock et al.'s index (stars and dashed line). Both scores are based on 198 antagonistic encounters.

was not found to have a significant influence on embryo mortality ($Z = -1.718$, $p = 0.086$).

Embryo survival was not positively linked to male dominance (DS: $r_s = -0.47$, $p = 0.18$; CBI: $r_s = -0.48$, $p = 0.17$; n always = 10) and male body length (Figure 2). The 95% confidence interval for the latter correlation is $-0.827 < r < 0.295$. A power analysis revealed that if the correlation between male length and offspring viability were at the upper limit of our calculated confidence interval, a minimal sample size of 88 males and more than 21,300 experimentally fertilized eggs would be necessary to demonstrate the effect at alpha ≤ 0.05 with our experimental methods and with a statistical power of at least 80%. Since this power analysis is for a possible correlation at the upper extreme of our observed confidence interval, we conclude that there is no or only a very weak positive correlation between male length and offspring survival. There was also no positive link between embryo survival and male age ($r = -0.50$, $n = 10$, $p = 0.14$) or weight ($r = -0.40$, $n = 10$, $p = 0.25$).

Second experiment

Male age ranged from 3 to 7 years (mean = 4.9 ± 1.2 (s.d.)). Average embryo survival was 42.1% (± 10.0 s.d.). We found again significant sire effects on embryo survival (Table 2), i.e. the males differed in genetic quality. We also found maternal effects on embryo survival, but no significant sire × dam interaction (Table 2). Embryo survival was again not significantly linked to male age ($r = -0.19$, $n = 13$, $p = 0.54$), body weight ($r = -0.22$, $n = 13$, $p = 0.69$), or body length (Figure 2). The correlation coefficient that describes the link between male body length and offspring viability in the second experiment (Figure 2) lies within the 95% confidence interval that we had obtained from the first experiment.

The statistical model in Table 2 explains a significant fraction of the total variance in embryo mortality. Dam effects include direct genetic effects, as well as maternal genetic and maternal environmental effects. Significant sire effects directly reveal variation in genetic quality. Assuming that epistatic genetic variance is of negligible

Table 1: The Influence of paternal, maternal and paternal × maternal interaction effects on embryo mortality in the 1st experiment.

		Model parameters						Likelihood ratio tests (LRT) with *reference model*		
Model	Effect tested	Random	Fixed	Number (k)	*ln L*	*AIC*	Δ*AIC*	χ^2	d.f.	p
reference model		F, M	T	3	-280.19	568.38				
full model	Male × Female	F, M, F × M	T	4	-280.19	570.38	2.00	0.00	1	1
female model	Male	F	T	2	-283.04	572.07	3.69	5.69	1	0.017
male model	Female	M	T	2	-403.65	813.30	244.92	246.92	1	<0.0001

Four logistic mixed effect models are compared to test if male (M), female (F), and male × female interaction (M × F) effects explain a significant part of the variance in embryo mortality (a binary response variable; egg number n = 2028). The random, fixed, and total number (k) of parameters are given for every model. The goodness of fit is given by the logarithm of the approximated likelihood (*ln L*) and the Akaikes information criterion (*AIC*). A measure to compare the quality of fit between two models is the difference of *AICs* (Δ*AIC*) between two models. The *reference model* explains our data best as the more complex *full model* does not significantly improve the qualtiy of fit (see Δ*AIC*; LRT), i.e. the male × female interaction effect did not explain a significant part of the variance in embryo mortality. The table therefore gives the differences in *AICs* between the *reference model* and the other models. Furthermore, likelihood ratio tests (LRT) between the *reference model* and the other models are given to test which parameter significantly improves the goodness of fit.

Table 2: Variance component analyses on embryo mortality in the 2nd experiment.

	SS	d.f.	F	p	σ^2 (% of total)
Sire	1.80	12	2.5	0.01	0.005023 (7.3%)
Dam	0.85	5	2.8	0.02	0.002818 (4.1%)
Sire × dam	3.58	60	1.0	0.53	0 (0%)
Total					0.068507 (100%)

Two-way ANOVA on embryo mortalities observed in the second breeding experiment when 13 males are crossed with 6 females in a full-factorial design and the embryos raised in 3 Petri dishes per sibship. Because the experimental set-up is fully balanced, results are based on EMS (Expected Mean Square). Sire, dam, and sire × dam interaction were random effects in the model. The negative estimate for the variance component of the interaction term is put to zero.

importance, the additive genetic variance can be calculated as four times the sire component of variance [16,39] and explains, in our second experiment, about 29.3% (4 × 0.005023/0.0.068507, see Table 2) of the total phenotypic variance in embryo mortality. The dam × sire effect can be used to estimate the non-additive genetic variance which here represents 0% of the total phenotypic variance in embryo mortality. The difference between the dam and sire component of variance is negative, i.e. the total maternal effect variance seems to be very low in the six females we used here.

We released 2443 hatchlings from this 2nd experiment into the streamlet. Nineteen juveniles could be caught back 20 months later (overall juvenile survival = 0.8%). Juvenile survival was not significantly linked to embryo survival (Figure 3a), and there was no significant positive relationship between juvenile survival and sire body length (Figure 3b), body weight ($-r_s = 0.14$, n = 13, p = 0.65), or age ($-r_s = 0.42$, n = 13, p = 0.16).

Discussion

Our first experiment shows that larger, heavier and older males were more dominant in male-male interactions than smaller, lighter and younger ones. This supports findings on other salmonid species where size was a good indicator for dominance status [9,29,40-43]. We have to leave it open whether body length, weight, or age is the better predictor for dominance rank as they were, as expected [44], highly correlated to each other. We used embryo survival until hatching as a main measure of sire 'good genes' because embryogenesis is a critical stage in offspring development (> 50% of offspring mortality normally happens at this stage under natural conditions [37,38]).

We found in both experiments that males differ in their offspring survival. We also tested for female effects on offspring survival. Such latter effects could be explained by differences in genetic quality among the females and/or by differences in egg quality. Variation in egg quality

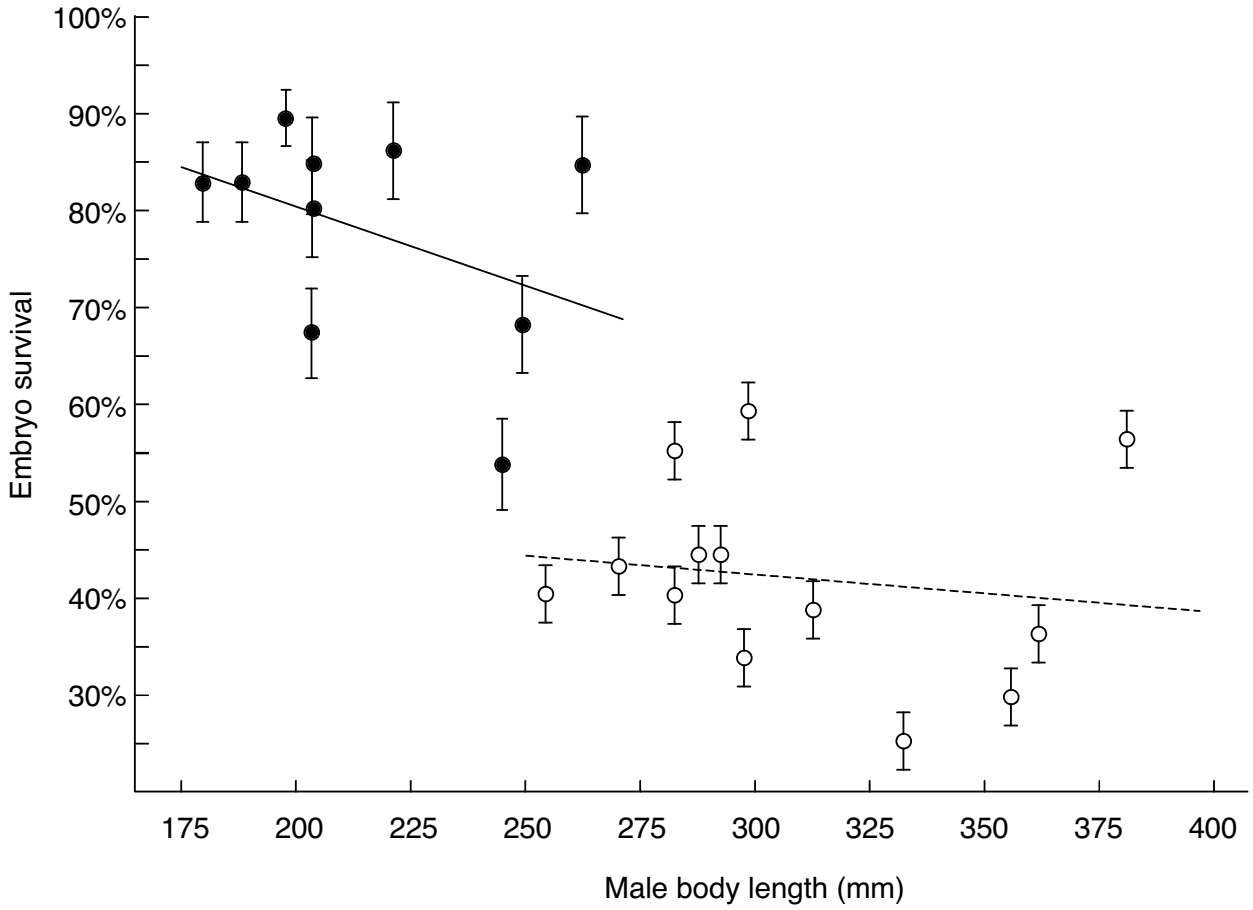


Figure 2

Embryo survival until hatching (means ± SE) versus male body length. The survival of visible embryos until hatching, i.e. excluding apparently non-fertilized eggs, for the first experiment (river "Müsche"; filled symbols and non-dashed regression line; Pearson's r = -0.41, n = 10, p = 0.24), and total embryo survival for the second experiment (river "Enziwigger", open symbols and dashed regression line; r = -0.15, n = 13, p = 0.63).

could be linked to female age, condition, and/or life history. Such differences in female investment are expected to be a crucial factor for egg survival in this development stage [45-47]. Accordingly, we found evidence for significant maternal effects on embryo survival in both experiments. Our experimental setup allowed us to control for these female effects. Analogously, males may differ in their sperm quality (e.g. sperm velocity, sperm longevity and spermatocrit), which could influence their fertilisation success [48,49]. We controlled for these potential differences by including only fertilised eggs in our measure of embryo survival in the first experiment. The second experiment was done with males that did not differ significantly in their fertilization ability (Wedekind, unpublished data). Therefore the sire effect that we found is directly linked to differences in male genetic quality and reveals additive genetic variation in embryo viability.

Although males differed in the viability of their embryos and hence in their genetic quality, male dominance or dominance-related characteristics were no indicators of 'good genes'. A power analysis shows that the chance of missing an existing correlation (type II error) is very low. In a second experiment, offspring viability was determined at two stages, as embryo survival in the laboratory and as juvenile survival in the field. In this second experiment we found again differences in male genetic quality but no significant connection between dominance traits and 'good genes'. Hence, we found no support for the hypothesis that dominant males are genetically superior. This seems to be in agreement with previous studies on species with parental care where no link between fathers' dominance and offspring viability was found [50,51], but the relative importance of variation in genetic quality and in parental care remains unclear in these studies.

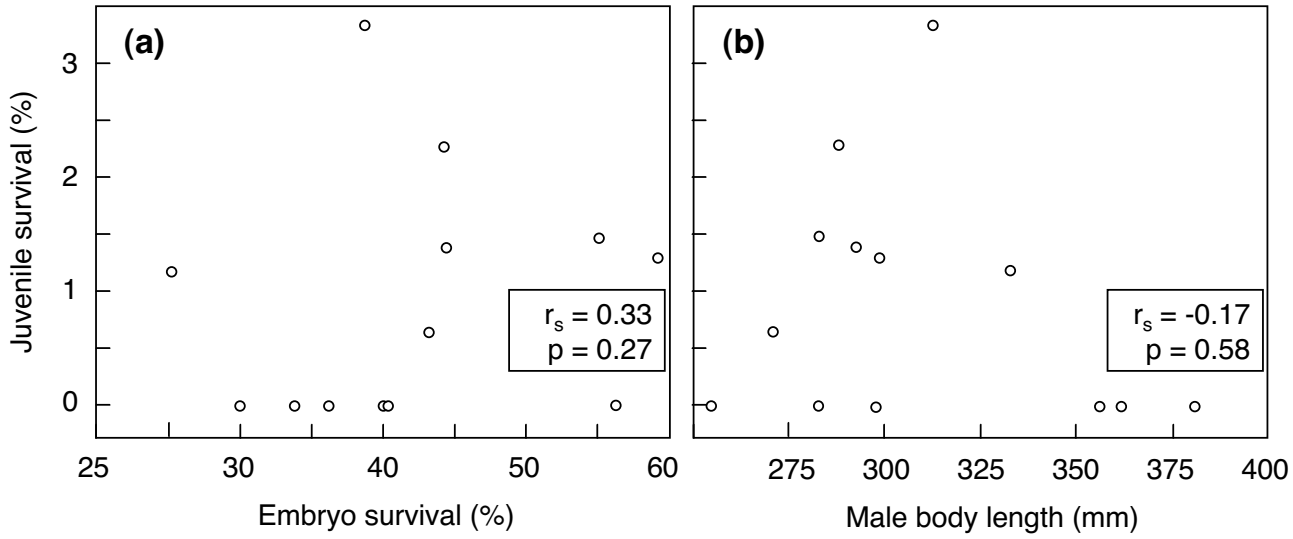


Figure 3

Juvenile survival in the field (means ± SE) versus embryo survival and male body length. Juvenile survival during 20 months as determined in the second experiment (total number of juveniles/total number of released hatchlings). The inserts give the Spearman rank order coefficients r_s and the two-tailed p-values.

Because male age, body length, body weight, and dominance are all strongly correlated in brown trout, we expect that older males will have a comparatively high reproductive success simply because they tend to be more dominant. However, according to our results females may not receive genetic benefits from mating with older males, contrary to some predictions from the literature [52-54]. It remains to be tested whether, for males that grow old, an original higher genetic quality is later in life reduced by an accumulation of germ-line mutations [52,55-57]. If so, larger and dominant males may still provide 'good genes' that may, however, only be revealed under certain environmental conditions. The observed embryo survival rates in our laboratory are high compared to more natural conditions [38], especially so in our first experiment, i.e. we incubated the embryos under conditions that may be less challenging than they would usually experience in the wild, giving lower quality embryos a higher survival chance. If so, it is possible that we missed some kinds of sire effects on embryo survival that may be revealed under more challenging conditions.

Conclusion

Some theory of sexual selection predicts that dominant and older males provide better genes to their offspring than subdominant and younger males. We found that larger, heavier, and older Brown trout male are indeed more dominant in male-male interactions, but females may not improve their offspring's genetic viability by mating with such males. Any advantage of mating with dominant males in brown trout may therefore be linked to a possibly increased fertilization success (but see [58-60]), potential benefits linked to the nest site [3], or to genetic advantages that are more context-dependent and not revealed at our experimental conditions [61] – even if we can demonstrate significant additive genetic variation for embryo viability under such conditions.

Methods

First experiment

We caught 10 males by electric fishing in the River Müsche (Kt. Bern, Switzerland) shortly before the breeding season. We then introduced them into an experimental channel (volume = 10 × 0.7 × 0.65 m, with gravel ground and several hiding places) in order to record their dominance behaviour. We recorded the winner and the loser of all antagonistic encounters (n = 198) during 8 observation days over a period of 32 days. The behaviour was recorded with 10 video surveillance cameras (CCD cam 1/3" SONY Super HAD, lens angle 78°, minimum illumination 0.05 Lux, Profiline®) linked to a MultiCam GV-1000 System (Ecoline®). Antagonistic encounters were defined as interactions between two males that resulted in one male leaving the spot of the interaction, or leaving it first. These interactions usually involved display behaviours, bites, and/or chases. To calculate dominance ranks, we used David's Score (DS) [62,63] and Clutton-Brock et al.'s index (CBI) [64], two methods that take the relative strength of the opponent into account.

After the observation period we recorded male body length, weight and age (determined from yearly growth rings on scale samples). The 10 males and 8 females from the same river were narcotised and the eggs and milt stripped individually into separate containers. The eggs of the females were equally distributed to 10 Petri dishes each. Ten µl of milt of one of the ten males' were added in such a way that all possible sibships (10 × 8 = 80) were produced (full-factorial breeding; [39]). Then, every Petri dish was half filled with water and shaken gently for about 5 seconds. Within the next ten hours all eggs were distributed (one egg per well in 2 ml of water) to 24-well Multiwell Plates (BD Falcon; nontreated polystyrene, flat bottom). The water we used for fertilisation and for incubation was standardized reconstituted water according to the OECD guideline for testing of chemicals [65]. The water volume per developing embryo corresponds to the ratio that [66] had used. Eggs of all 80 combinations (n = 2028 with 23.56 ± 10.24 (mean ± std deviation) eggs per combination) were incubated at one of two incubation temperatures (6.9°C and 8.9°C). Water was not changed during the experiment. Egg viability was measured as the survival of visible embryos until hatching, i.e. we excluded apparently non-fertilized eggs and embryos that died before they were visible under a stereomicroscope (Olympus SZX9).

Second experiment

Brown trout were collected from their natural spawning place in River Enziwigger (Kt. Luzern, Switzerland) in November by electro-fishing. Thirteen mature males were measured for length and weight, and their age was determined from yearly growth rings on scales sampled below the adipose fin near the lateral line. Their milt was stripped for *in vitro* fertilization of the eggs of six females of the same population in again a full-factorial set-up (North Carolina II design). We used 20 µl milt per 80–100 eggs (see [66], for the detailed methods). The resulting embryos were reared in 3 separate Petri dishes per sibship in 50 ml sand-filtered lake water at 4.7°C (mean number of eggs per Petri dish: 20.4 ± 14.1 s.d.). From day 46 after fertilization on, inviable embryos and hatched larvae were recorded and carefully removed from the Petri dishes with a plastic spoon (in regular intervals of about 10 days each). Water was exchanged twice (at day 76 and day 88 after fertilization). Embryo viability was determined for each Petri dish as the number of hatchlings per total number of eggs.

Alevins were kept in darkness in running water at 7–8°C until all embryos had hatched and most alevins had nearly used up their yolk sac, i.e. until day 131 after fertilization. We then released all fish plus some additional ones (Evanno, unpublished data) into a 600 m long streamlet that is confined by two waterfalls. This structured streamlet has a width of up to half a meter and an average depth of about 10 cm. We removed all trouts by electrofishing and released our fish by carefully distributing them over the full length of the streamlet during a period when water discharge was low and not obviously affecting the larvae. We caught the fish back 20 months later by electrofishing. DNA was extracted from fin clips using the DNeasy Tissue kit (Qiagen) following manufacturer instructions. Eight microsatellite markers were used to determine paternity: *Mst85* [67], *Mst543AE*, *BS131*, *T3-13* [68], *AETG1* [69], *Ssosl417* [70], *Ssa 171* [71] and *Str58* [72]. PCR reactions were performed in 10 µL reaction mixtures containing 2.5 µL of DNA template, 1 × PCR buffer (Qiagen), 1.5–2 mM MgCl2, 0.2 mM dNTPs, 0.5 µM of each primer and 0.25 units of Taq DNA polymerase (Applied Biosystems or Qiagen). PCR profile consisted in 30 iterations of 95°C for 30 s, 50°C (*Mst85*, *BS131*), 55°C (*Ssa 171*, *Ssosl417*, *Str58*), 58°C (*Mst543AE*) or 60°C (*AETG1*, *T3-13*) for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. PCR products were analyzed with an ABI 3100 automated DNA sequencer (Applied Biosystems) using the Genemapper software (Applied Biosystems). Paternity was established using the CERVUS program [73].

Statistical analyses

In the first experiment where embryos were raised singly, we analysed embryo mortality as binary response variable with logistic mixed-effect models (every embryo as one independent data point; dead before hatching or hatched). We entered rearing temperature as fixed effect, and parent identity as random male, female, and male × female interaction effects. To test whether male, female and male × female interaction effects explain a significant part of the variance in offspring mortality, we fitted a "*full model*" (including all effects), a "*reference model*" (including temperature, male and female effects only), a "*female model*" (including temperature and female effects only) and a "*male model*" (including temperature and male effects only) and tested if the goodness of fit between models differed. The goodness of fit is given both by the logarithm of the approximated likelihood (*ln L*) and by the Akaikes information criterion (*AIC*)[74]. The latter is based on the *ln L* but punishes for the number of included parameters (k) and is calculated as $AIC_i = -2\ ln\ L_i + 2\ k_i$. The *AIC* favours models that have a high goodness of fit with the smallest number of entered parameters. To test if models differ in their goodness of fit, we compared the models with likelihood ratio tests (LRT), calculated as: $\chi^2 = 2(ln\ L_1 - ln\ L_2)$. The degree of freedom is the difference in number of free parameters in the two models. The test statistic is then evaluated under the assumption of asymptotic convergence to a χ^2 distribution. A second measure that compares the quality of fit between two models is given as the difference of *AICs* (Δ*AIC*), which is here

calculated as $\Delta_i AIC = AIC_i - AIC_{\text{Reference Model}}$. An $\Delta AIC \leq 2$ indicates substantial support that the two models do not differ in the quality of fit, values between 4 and 10 indicate some support that they differ in the quality of fit, and $\Delta AIC \geq 10$ provide much support that the models differ in their quality of fit [75,76]. Analyses were done with the R software [77] and we used the lme4 package for logistic mixed effect model analyses [78].

Embryo mortality in the second experiment was determined for batches of embryos each. We could therefore calculate a two-way ANOVA with the sire and dam identity and sire × dam interaction as random effects and mortality per Petri dish (square-root arcsin transformed) as response variable. This analysis was done with JMP In statistical package JMP V [79]. Graphical inspection of the juvenile survival data suggested that the assumptions of parametric statistics might be significantly violated and hence non-parametric statistics (Spearman rank order correlation coefficients r_s) was used. All p-values are two-tailed.

Authors' contributions

AJ, AB and CW conceived the first experiment and did the experimental breeding. AJ determined the dominance scores. AJ and AB recorded embryo mortality and hatching date. CW and RM conceived the second experiment and did the experimental breeding, CW recorded embryo mortality. RM, CW, and AJ released the hatchlings into the streamlet and caught them back 20 months later. GE did the molecular analyses on the parents and the juveniles of the second experiment. SN, AJ and CW analysed the data. AJ and CW wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank P. Amrein, M. Flück, U. Gutmann, and H. Walther for providing the fish and for various kinds of support, M. Bia and A. Steffen for assistance, and D. Bittner, A. Champigneulle, P. Fitze, R. Gingold, C. Kropf, T. Pfeiffer, B. von Siebenthal, D. Urbach, and the anonymous referees for discussion and useful comments. The study was supported by the Swiss National Science Foundation.

References

1. Garant D, Dodson JJ, Bernatchez L: **Differential reproductive success and heritability of alternative reproductive tactics in wild Atlantic salmon (*Salmo salar L.*).** *Evolution* 2003, **57(5):**1133-1141.
2. Andersson MB: **Sexual selection.** New Jersey , Princeton University Press; 1994.
3. Blanchfield PJ, Ridgway MS: **The cost of peripheral males in a brook trout mating system.** *Anim Behav* 1999, **57:**537-544.
4. Wiley RH: **Territoriality and non-random mating in the sage grouse *Centrocercus urophasiasus*.** *Anim Behav Monogr* 1973, **6:**87-169.
5. Dewsbury DA: **Dominance rank, copulatory-behavior, and differential reproduction.** *Q Rev Biol* 1982, **57(2):**135-159.
6. Gibson RM: **Bivariate versus multivariate analyses of sexual selection in Red deer.** *Anim Behav* 1987, **35:**292-293.
7. Mjolnerod IB, Fleming IA, Refseth UH, Hindar K: **Mate and sperm competition during multiple-male spawnings of Atlantic salmon.** *Can J Zool* 1998, **76(1):**70-75.
8. Dickerson BR, Brinck KW, Willson MF, Bentzen P, Quinn TP: **Relative importance of salmon body size and arrival time at breeding grounds to reproductive success.** *Ecology* 2005, **86(2):**347-352.
9. Kitano S: **Size-related factors causing individual variation in seasonal reproductive success of fluvial male Dolly Varden (*Salvelinus malma*).** *Ecol Freshw Fish* 1996, **5(2):**59-67.
10. Bekkevold D: **Male size composition affects male reproductive variance in Atlantic cod *Gadus morhua L.* spawning aggregations.** *J Fish Biol* 2006, **69(3):**945-950.
11. Kokko H: **Treat 'em mean, keep 'em (sometimes) keen: evolution of female preferences for dominant and coercive males.** *Evol Ecol* 2005, **19(2):**123-135.
12. Candolin U: **Male-male competition ensures honest signaling of male parental ability in the three-spined stickleback (*Gasterosteus aculeatus*).** *Behav Ecol Sociobiol* 2000, **49(1):**57-61.
13. Candolin U: **Increased signalling effort when survival prospects decrease: male-male competition ensures honesty.** *Anim Behav* 2000, **60:**417-422.
14. Qvarnstrom A, Griffith SC, Gustafsson L: **Male-male competition and parental care in collared flycatchers (*Ficedula albicollis*): an experiment controlling for differences in territory quality.** *Proc R Soc Lond B Biol Sci* 2000, **267(1461):**2547-2552.
15. Bisazza A, Marconato A, Marin G: **Male competition and female choice in *Padogobius martensi* (Pisces, Gobiidae).** *Anim Behav* 1989, **38:**406-413.
16. Neff BD, Pitcher TE: **Genetic quality and sexual selection: an integrated framework for good genes and compatible genes.** *Mol Ecol* 2005, **14(1):**19-38.
17. Qvarnstrom A, Forsgren E: **Should females prefer dominant males?** *Trends Ecol Evol* 1998, **13(12):**498-501.
18. Wedekind C: **Sexual selection and life-history decisions: implications for supportive breeding and the management of captive populations.** *Conserv Biol* 2002, **16:**1204-1211.
19. Kirkpatrick M, Ryan MJ: **The Evolution of Mating Preferences and the Paradox of the Lek.** *Nature* 1991, **350(6313):**33-38.
20. Petrie M, Roberts G: **Sexual selection and the evolution of evolvability.** *Heredity* 2007, **98(4):**198-205.
21. Tomkins JL, Radwan J, Kotiaho JS, Tregenza T: **Genic capture and resolving the lek paradox.** *Trends Ecol Evol* 2004, **19(6):**323-328.
22. Moller AP, Alatalo RV: **Good-genes effects in sexual selection.** *Proc R Soc Lond B Biol Sci* 1999, **266(1414):**85-91.
23. Keyser AJ, Hill GE: **Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks.** *Behav Ecol* 2000, **11(2):**202-209.
24. Milinski M, Bakker TCM: **Female sticklebacks use male coloration in mate choice and hence avoid parasitized males.** *Nature* 1990, **344(6264):**330-333.
25. Petrie M: **Improved growth and survival of offspring of peacocks with more elaborate trains.** *Nature* 1994, **371(6498):**598-599.
26. Petrie M, Halliday T: **Experimental and natural changes in the peacocks (*Pavo cristatus*) train can affect mating success.** *Behav Ecol Sociobiol* 1994, **35(3):**213-217.
27. Wedekind C, Müller R, Spicher H: **Potential genetic benefits of mate selection in whitefish.** *J Evol Biol* 2001, **14:**980-986.
28. Welch AM, Semlitsch RD, Gerhardt HC: **Call duration as an indicator of genetic quality in male gray tree frogs.** *Science* 1998, **280(5371):**1928-1930.
29. Fleming IA: **Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids.** *Can J Fish Aquat Sci* 1998, **55:**59-76.
30. Garant D, Dodson JJ, Bernatchez L: **A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar L.*).** *J Hered* 2001, **92(2):**137-145.
31. Largiader CR, Estoup A, Lecerf F, Champigneulle A, Guyomard R: **Microsatellite analysis of polyandry and spawning site competition in brown trout (*Salmo trutta* L.).** *Genet Sel Evol* 2001, **33:**S205-S222.
32. Quinn TP, Foote CJ: **The effects of body-size and sexual dimorphism on the reproductive behavior of Sockeye-salmon, *Oncorhynchus nerka*.** *Anim Behav* 1994, **48(4):**751-761.

33. Quinn TP, Hendry AP, Buck GB: **Balancing natural and sexual selection in sockeye salmon: interactions between body size, reproductive opportunity and vulnerability to predation by bears.** *Evol Ecol Res* 2001, **3(8):**917-937.
34. Weir LK, Hutchings JA, Fleming IA, Einum S: **Dominance relationships and behavioural correlates of individual spawning success in farmed and wild male Atlantic salmon, *Salmo salar*.** *J Anim Ecol* 2004, **73(6):**1069-1079.
35. de Gaudemar B, Bonzom JM, Beall E: **Effects of courtship and relative mate size on sexual motivation in Atlantic salmon.** *J Fish Biol* 2000, **57(2):**502-515.
36. Petersson E, Jarvi T, Olsen H, Mayer I, Hedenskog M: **Male-male competition and female choice in brown trout.** *Anim Behav* 1999, **57:**777-783.
37. Blanchfield PJ, Ridgway MS: **The relative influence of breeding competition and habitat quality on female reproductive success in lacustrine brook trout (*Salvelinus fontinalis*).** *Can J Fish Aquat Sci* 2005, **62(12):**2694-2705.
38. Bradford MJ: **Comparative review of Pacific salmon survival rates.** *Can J Fish Aquat Sci* 1995, **52(6):**1327-1338.
39. Lynch M, Walsh B: **Genetics and analysis of quantitative traits.** Sunderland, Massachusetts , Sinauer Associates Inc; 1998.
40. Foote CJ, Brown GS, Wood CC: **Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*.** *Can J Fish Aquat Sci* 1997, **54(8):**1785-1795.
41. Fleming IA, Gross MR: **Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): Measures of natural and sexual selection.** *Evolution* 1994, **48(3):**637-657.
42. Keenleyside MHA, Dupuis HMC: **Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*).** *Can J Zool* 1988, **66(1):**262-265.
43. Esteve M: **Observations of spawning behaviour in Salmoninae: *Salmo, Oncorhynchus* and *Salvelinus*.** *Rev Fish Biol Fish* 2005, **15(1-2):**1-21.
44. Weatherhead PJ, Dufour KW, Lougheed SC, Eckert CG: **A test of the good-genes-as-heterozygosity hypothesis using red-winged blackbirds.** *Behav Ecol* 1999, **10(6):**619-625.
45. Einum S, Fleming IA: **Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*).** *Evolution* 2000, **54(2):**628-639.
46. Einum S, Hendry AP, Fleming IA: **Egg-size evolution in aquatic environments: does oxygen availability constrain size?** *Proc R Soc Lond B Biol Sci* 2002, **269(1507):**2325-2330.
47. Einum S, Kinnison MT, Fleming IA: **Evolution of egg size and number.** In *Evolution illuminated; Salmon and their relatives* Edited by: Hendry AP, Stearns SC. New York , Oxford University Press; 2004:126-153.
48. Wedekind C, Rudolfsen G, Jacob A, Urbach D, Muller R: **The genetic consequences of hatchery-induced sperm competition in a salmonid.** *Biol Conserv* 2007, **137(2):**180-188.
49. Snook RR: **Sperm in competition: not playing by the numbers.** *Trends Ecol Evol* 2005, **20(1):**46-53.
50. Verhulst S, Salomons HM: **Why fight? Socially dominant jackdaws, *Corvus monedula*, have low fitness.** *Anim Behav* 2004, **68:**777-783.
51. Wong BBM: **Superior fighters make mediocre fathers in the Pacific blue-eye fish.** *Anim Behav* 2004, **67:**583-590.
52. Brooks R, Kemp DJ: **Can older males deliver the good genes?** *Trends Ecol Evol* 2001, **16(6):**308-313.
53. Kokko H, Lindstrom J: **Evolution of female preference for old mates.** *Proc R Soc Lond B Biol Sci* 1996, **263(1376):**1533-1538.
54. Manning JT: **Choosy females and correlates of male age.** *J Theor Biol* 1985, **116(3):**349-354.
55. Garant D, Fontaine PM, Good SP, Dodson JJ, Bernatchez L: **The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*).** *Evol Ecol Res* 2002, **4(4):**537-549.
56. Hansen TF, Price DK: **Good genes and old age: Do old mates provide superior genes?** *J Evol Biol* 1995, **8(6):**759-778.
57. Price DK, Hansen TF: **How does offspring quality change with age in male Drosophila melanogaster?** *Behav Genet* 1998, **28(5):**395-402.
58. Berejikian B, Tezak EP: **Male size effects on fertilization success: lack of evidence in chinook salmon spawning under experimental conditions.** *Environ Biol Fishes* 2005, **72(3):**235-240.
59. Dickerson BR, Willson MF, Bentzen P, Quinn TP: **Size-assortative mating in salmonids: negative evidence for pink salmon in natural conditions.** *Anim Behav* 2004, **68:**381-385.
60. Figenschou L, Rudolfsen G, Folstad I: **Female Arctic charr do not show apparent benefits from exposing their eggs to sperm from dominant males.** *J Fish Biol* 2007, **71:**284-289.
61. Welch AM: **Genetic benefits of a female mating preference in gray tree frogs are context-dependent.** *Evolution* 2003, **57(4):**883-893.
62. David HA: **The method of paired comparisons.** London , Charles Griffin; 1988.
63. Gammell MP, De Vries H, Jennings DJ, Carlin CM, Hayden TJ: **David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index.** *Anim Behav* 2003, **66:**601-605.
64. Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE: **Logical stag - adaptive aspects of fighting in red deer (*Cervus elaphus L*).** *Anim Behav* 1979, **27(FEB):**211-225.
65. OECD: **Guideline 203: fish, accute toxicity test.** Organisation for Economic Co-operation and Development. ; 1992.
66. Wedekind C, Müller R: **The experimental rearing of large salmonid eggs in Petri dishes.** *Funct Ecol* 2004, **18(1):**138-140.
67. Presa P, Guyomard R: **Conservation of microsatellites in three species of salmonids.** *J Fish Biol* 1996, **49(6):**1326-1329.
68. Estoup A, Rousset F, Michalakis Y, Cornuet JM, Adriamanga M, Guyomard R: **Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*).** *Mol Ecol* 1998, **7(3):**339-353.
69. Estoup A, Presa P, Krieg F, Vaiman D, Guyomard R: **(Ct)(N) and (Gt)(N) Microsatellites - a New Class of Genetic-Markers for Salmo-Trutta L (Brown Trout).** *Heredity* 1993, **71:**488-496.
70. Slettan A, Olsaker I, Lie O: **Atlantic Salmon, Salmo-Salar, Microsatellites at the Ssosl25, Ssosl85, Ssosl311, Ssosl417 Loci.** *Anim Genet* 1995, **26(4):**281-282.
71. Oreilly PT, Hamilton LC, McConnell SK, Wright JM: **Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites.** *Can J Fish Aquat Sci* 1996, **53(10):**2292-2298.
72. Poteaux C, Bonhomme F, Berrebi P: **Microsatellite polymorphism and genetic impact of restocking in Mediterranean brown trout (*Salmo trutta L.*).** *Heredity* 1999, **82:**645-653.
73. Marshall TC, Slate J, Kruuk LEB, Pemberton JM: **Statistical confidence for likelihood-based paternity inference in natural populations.** *Mol Ecol* 1998, **7(5):**639-655.
74. Akaike H: **New look at the statistical model identification.** *IEEE Trans Aut Contr* 1974, **AC-19(6):**716-723.
75. Sullivan J, Joyce P: **Model selection in phylogenetics.** *Annu Rev Ecol Evol Syst* 2005, **36:**445-466.
76. Burnham KP, Anderson DR: **Multimodel inference - understanding AIC and BIC in model selection.** *Sociol Method Res* 2004, **33(2):**261-304.
77. R Development Core Team: **R: A language and environment for statistical computing.** 2006 [http://www.R-project.org]. Vienna, Austria , R Foundation for Statistical Computing
78. Bates D, Sarkar D: **lme4: Linear mixed-effects models using S4 classes.** 2007:R package version 0.9975-13.
79. JMP V: **JMP.** [http://www.jmp.com].

PROCEEDINGS OF THE ROYAL SOCIETY B

FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2008.0072
Published online

Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers

Claus Wedekind[1,2,*], Alain Jacob[1], Guillaume Evanno[1,†], Sébastien Nusslé[1] and Rudolf Müller[2]

[1]*Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland*
[2]*Eawag, Swiss Federal Institute of Aquatic Science and Technology, 6047 Kastanienbaum, Switzerland*

'Good-genes' models of sexual selection predict significant additive genetic variation for fitness-correlated traits within populations to be revealed by phenotypic traits. To test this prediction, we sampled brown trout (*Salmo trutta*) from their natural spawning place, analysed their carotenoid-based red and melanin-based dark skin colours and tested whether these colours can be used to predict offspring viability. We produced half-sib families by *in vitro* fertilization, reared the resulting embryos under standardized conditions, released the hatchlings into a streamlet and identified the surviving juveniles 20 months later with microsatellite markers. Embryo viability was revealed by the sires' dark pigmentation: darker males sired more viable offspring. However, the sires' red coloration correlated negatively with embryo survival. Our study demonstrates that genetic variation for fitness-correlated traits is revealed by male colour traits in our study population, but contrary to predictions from other studies, intense red colours do not signal good genes.

Keywords: salmonid; genetic load; good-genes sexual selection; offspring survival; heritability of colour traits

1. INTRODUCTION

Some 'good-genes' models of sexual selection predict that attractive males or males that are superior competitors in intrasexual selection are of high genetic quality and hence offer indirect genetic benefits to females (Neff & Pitcher 2005). Empirical tests of such models need to control for potentially confounding effects of, for example, differential female investment into embryos and juveniles (Parker 2003). Therefore, the ideal species to test for genetic effects of sexual selection are those with no parental care, external fertilization and large family size, i.e. some frogs (Welch *et al.* 1998) and some fish species (e.g. Wedekind *et al.* 2001, 2004; Rudolfsen *et al.* 2005; Pitcher & Neff 2006). However, it is still unclear whether colours can be linked to genetic quality in such species.

Animals often use colours to advertise their quality and to attract mates. Two main groups have been discussed as candidates in this context (McGraw 2005; Griffith *et al.* 2006): carotenoid-based yellow or red colours and melanin-based brown, black and grey colours. The reliability of the signals they produce is assumed to be given by the costs of colour production and/or maintenance (Grafen 1990). Various non-exclusive types of costs have been discussed (Hadfield & Owens 2006) as follows: (i) the pigments are costly to acquire, e.g. because they are rare or owing to costly physiological handling (metabolism or transport), (ii) the pigments are required by other physiological processes, e.g. for immune functioning, or (iii) the pigments are toxic or the colours are dangerous, e.g. because they enhance conspicuousness to predators or they signal a social status that may need to be defended.

In vertebrates, carotenoids that are responsible for yellow and red colours have to be obtained through the diet. They may therefore reliably signal foraging quality (Olson & Owens 1998) or foraging strategy (Bakker *et al.* 1997). Such colours have often been investigated in sexual selection studies because they are usually conspicuous and based on pigments that also have antioxidant and immunoregulatory properties (McGraw 2005; Peters 2007). Hence, by using these pigments, the signaller may reveal its superior health and vigour to potential mates (McGraw & Ardia 2003). In the three-spined stickleback (*Gasterosteus aculeatus*), for example, males use conspicuous red colours to attract females (Milinski & Bakker 1990; Bakker & Mundwiler 1994). These colours are based on at least three different carotenoids (Wedekind *et al.* 1998) that are known to be important in immune function (Krinsky 1993). Accordingly, red colours in these fish reveal good condition (Frischknecht 1993; Barber *et al.* 2000) and high resistance against parasite infection (Milinski & Bakker 1990; Barber *et al.* 2001), and females benefit from preferring red males especially when males are energy constrained during their paternal care (Candolin 2000).

In species without paternal care, i.e. where males only provide genes to their offspring, the role of carotenoid-based colours in signalling and mate choice is less clear. In Arctic charr (*Salvelinus alpinus*), for example, first

*Author and address for correspondence: Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland (claus.wedekind@unil.ch).
†Present address: INRA, UMR985, Ecology and Health of Ecosystems, 35000 Rennes, France.

Received 17 January 2008
Accepted 10 April 2008

observations found a link between red colours and immune function (Skarstein & Folstad 1996), but these colours do not seem to be useful indicators of health and vigour or resistance against infection: Skarstein *et al.* (2005) even found a positive correlation between red coloration and parasite intensity, and Masvaer *et al.* (2004) reported a positive link between red coloration and milt characteristics that indicate low dominance in male–male interaction at the spawning place (Rudolfsen *et al.* 2006). In guppies (*Poecilia reticulata*), another fish with no paternal care, variation in carotenoid-based colour traits can be linked to infections and reactions of the immune system (Grether *et al.* 2004; Kolluru *et al.* 2006), and females have been found to prefer males with larger amounts of carotenoids in their orange spots (Grether 2000). However, female responsiveness to male colours varies with carotenoid availability (Grether *et al.* 2005) and age (Kodric-Brown & Nicoletto 2001), and females are also reported to prefer larger males to brightly coloured ones (Houde 1997).

In contrast to carotenoid-based colours, melanin-based colours can be synthesized by the animal itself (e.g. in specialized organelles called melanosomes; Sugimoto 2002) and the synthesis is under strong genetic control (Majerus & Mundy 2003). Melanin-based colours may have therefore been less obvious candidates for reliable signals of genetic quality, but it has recently been recognized that significant relationships exist between melanin-based pigmentation and overall health and vigour (Roulin 2004*a*; McGraw 2005). In birds, melanin-based traits are frequently linked to fitness traits (reviewed by Roulin (2004*b*); recent examples include Bize *et al.* (2006), Fargallo *et al.* (2007) and Roulin (2007)). These links could be due to the antioxidant activity of melanin (McGraw 2005) or its involvement in calcium, zinc and iron metabolism and in the maintenance of body structures (Niecke *et al.* 2003; Roulin *et al.* 2006; McGraw 2007). In addition, melanin plays important roles in the developmental pathways of various functions and may therefore be an indicator of homeostasis (Badyaev & Young 2004; Roulin 2004*a*). Finally, although melanin-based colours often function as camouflage, some melanin-based signals may make the signaller more conspicuous in some environments (Smith *et al.* 2004), and only animals in good health and vigour may be able to pay the costs of being conspicuous (Kodric-Brown 1998).

The brown trout is a salmonid with external fertilization and no paternal care, and with migratory and resident forms. The resident form (*Salmo trutta* resident morph) develops brown, black and red spots on the body sides and the adipose fin. The red spots on the skin and the adipose fin contain large quantities of different carotenoids (xanthophylls and astacene esters) and much more so than any other tissue studied (Steven 1948). The migratory forms of brown trout are usually larger and therefore expected to be more dominant at the spawning place (Jacob *et al.* 2007); they have dark spots but usually no red spots when they arrive at the spawning place. We chose a resident population that has not been supplemented by hatchery fish since at least 10 years. We tested whether males differ in their genetic quality, determined as their offspring viability, and if so, whether male colour traits reveal genetic quality. We also tested for heritabilities of colour traits by comparing 2-year-old juveniles with their fathers.

2. MATERIAL AND METHODS

Brown trout were collected from their natural spawning place in River Enziwigger (near Willisau, Switzerland) in November by electrofishing. Fifteen mature males were narcotized, measured for size and weight and their milt stripped for the *in vitro* fertilization of the eggs of one large female of the same population. The eggs of this one female were about equally distributed to 15 Petri dishes and 20 µl of milt were added each and activated with sand-filtered lake water (8°C), following the methods of Wedekind & Müller (2004). The resulting embryos were reared in four separate Petri dishes per sibship in 50 ml sand-filtered lake water at 4.7°C (mean number of eggs per Petri dish: 22.3 ± 12.0 s.d.; three unfertilized eggs were earlier discarded).

The males were individually marked and kept together in a large tank at approximately 8°C for another breeding experiment 14 days later (Jacob *et al.* 2007). They were then killed with a sharp blow on their head and photographed from both sides under standardized conditions with a digital camera (Nikon E995, 2048×1536 pixels; figure 1*a*,*b*). Scale samples were taken from below the adipose fin near the lateral line to estimate the age of the fish from yearly grow rings (Rifflart *et al.* 2006).

From day 60 after fertilization onwards, non-viable embryos and hatched larvae were recorded and carefully removed from the Petri dishes with a plastic spoon (in regular intervals of approx. 10 days each). Alevins were kept in darkness in running water at 7–8°C until all embryos had hatched and most alevins had nearly used up their yolk sac, i.e. until day 131 after fertilization. We then released all fish plus some additional ones from two other studies (Jacob *et al.* 2007; Evanno *et al.* submitted) into a 600 m long semi-natural, structured streamlet near Willisau that is typical for the streamlets in the region and that is confined by physical barriers. The streamlet has a width of up to 0.5 m and an average depth of approximately 10 cm. We removed all present trout by electrofishing and released our fish by carefully distributing them over the full length of the streamlet during a period when water discharge was low and not obviously affecting the larvae. We caught the fish back 20 months later again by electrofishing, measured their weight and length and took standard photos from both sides (Olympus C77OUZ, 2288×1712 pixels; figure 1*c*). DNA was extracted from fin clips, and eight microsatellite markers (Mst85, Mst543AE, BS131, T3-13, AETG1, Sssosl417, Ssa171 and Str58) were used for parental assignment following the procedure described by Jacob *et al.* (2007). Paternity was established by exclusion using the Cervus program (Marshall *et al.* 1998). All inferred parent–offspring pairs were checked for the absence of mismatching loci.

The photos of the parental and juvenile fish were used to count the number of red and brown or black spots. Further colour analyses were done on TIFF files in the open-access software ImageJ (http://rsb.info.nih.gov/ij/). We used a mode that defines hue, saturation and brightness for every pixel of the image (the 'HSB mode'). Hue is given as an angle on a continuous circular scale from 0° to 360°, with 0° for red, 60° for yellow, 120° for green, 180° for cyan, 240° for blue and 300° for magenta. Saturation is the purity of the colour from 0% (grey) to 100% (fully saturated colour), and brightness is the relative lightness or darkness of the colour from 0% (black) to 100% (white). In ImageJ, all three values are converted to a scale from 0 to 255, and thus every pixel is defined by three values within this range. To obtain the

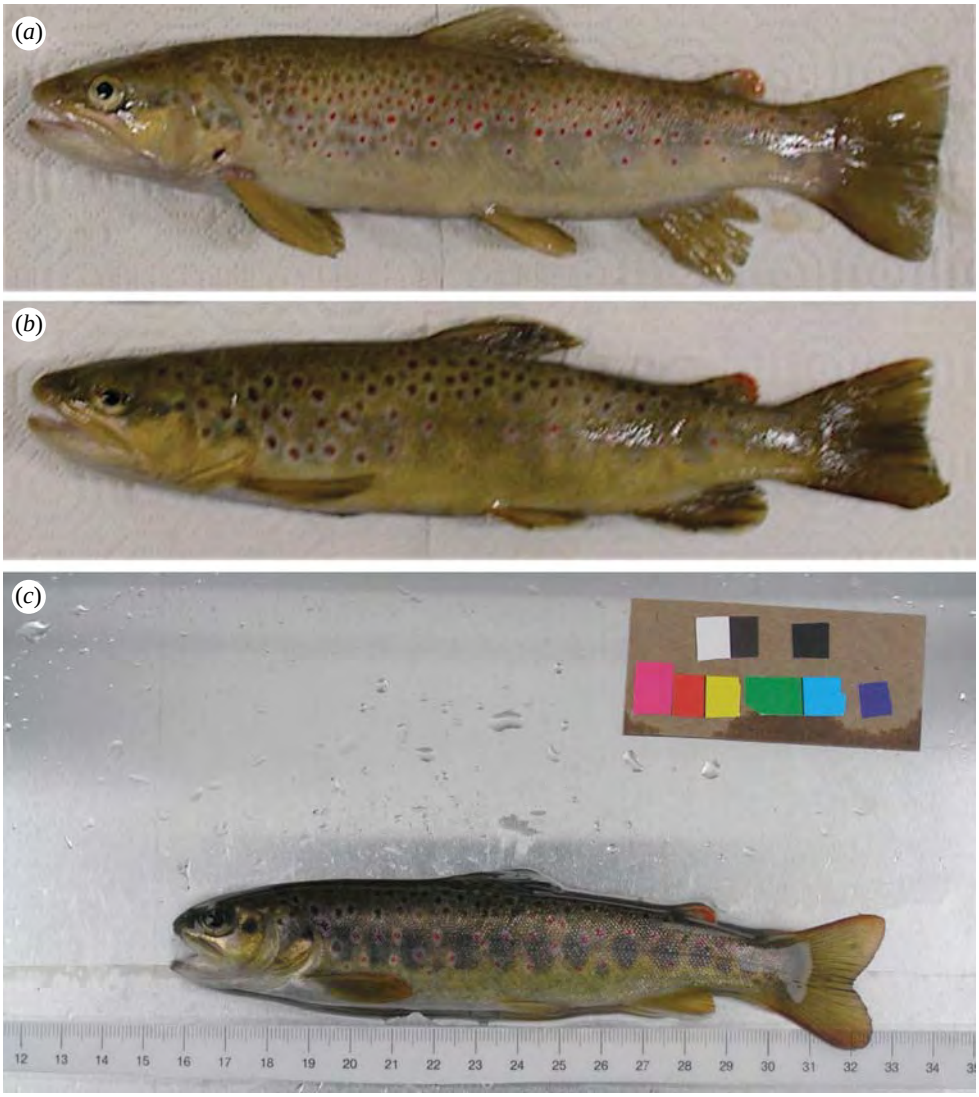


Figure 1. Size-standardized photos of (*a*) the male that received the highest values for red coloration but whose embryos had the lowest survival and (*b*) the male with the highest embryo survival. (*c*) Standard-distance photo of a typical juvenile that was caught back after 20 months in the wild. The reference colour patches are taken from Kodak Color Separation Guide and Grey Scale.

measurements of the red coloration of our fish, we defined *a priori* a range of reddish hue values (225°–255° and 0°–20°). We then counted all the pixels within this range to determine a fish's overall redness as the total red area on the body sides and the adipose fin divided by the total area of the body sides and the adipose fin. The average modal hue and saturation of the red coloration, i.e. the most frequent hue and saturation values within the area that was perceived as red, were used as a qualitative measure of the red pigmentation. We also determined the average modal grey value on both body sides, i.e. the most frequent grey value as a measure of overall dark pigmentation. Our methods did not, however, allow us to measure colours in the ultraviolet spectrum. See Stevens *et al.* (2007) for a discussion of colour measurements from digital photos. Figure 1 indicates the range of observed male phenotypes. The analogous measures were taken from the standard photos of the 2-year-old offspring.

Statistical analyses were done in JMP v. 6.0 (www.jmp.com) or the open-access software R (www.r-project.org; see also Bates 2005). Pearson's correlation coefficients r are used when graphical inspection of the data suggested that the model assumptions of this statistics were not notably violated, otherwise Spearman's rank-order correlation coefficients r_s are used. Mixed-effects logistic models were used to test for sire effects on offspring viability. To avoid potentially confounding Petri-dish effects, we excluded all Petri dishes that could be considered as outliers, i.e. they were linked to embryo mortalities that were 1.5 times larger than the interquartile of the respective half-sib family (4 out of 60 Petri dishes). The sire effect was then assessed in two tests: first, a log-likelihood ratio test between a reference model (including both Petri dish and sire effect) and a reduced model (with only one of the two parameters); second, the generation of a log-likelihood distribution of the model under the null hypothesis by random permutation ($n=1000$) and a rank comparison of the observed likelihood with this distribution.

3. RESULTS

Fertilization success was close to 100% for all males (only three out of in total 1454 eggs did not seem to contain an embryo). Embryos were clearly visible on day 60 after fertilization, with no apparent mortality until then. Total embryo survival until hatching was 86.9% (median 90%, range 20–100% per Petri dish). Males

Table 1. Sire effects on embryo survival. (Three mixed-effects logistic models to test whether sire effects explain a significant part of the variance in embryo survival (a binary response variable; egg number $n=1290$). Model parameters are random effects. The goodness of fit is given by the logarithm of the approximated likelihood (log L) and the Akaike information criterion (AIC). A measure to compare the quality of fit between two models is the difference in AICs between two models. Likelihood ratio tests between the reference model and the other models are used to test which parameter significantly improves the goodness of fit.)

					likelihood ratio test against the reference model		
model	effect tested	model parameters	log L	AIC	χ^2	d.f.	p
reference model		Petri dish, sire	−383.79	773.58			
environmental model	sire	Petri dish	−387.15	778.31	6.73	1	0.0095
sire model	Petri dish	sire	−383.79	771.58	<0.01	1	1

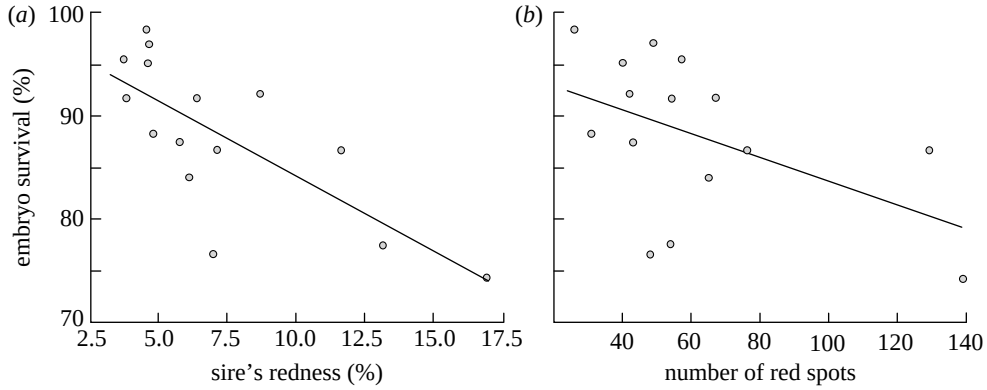


Figure 2. Male red colour characteristics versus embryo survival. Total embryo survival until hatching versus (*a*) overall redness, calculated as the relative red area on the body sides and the adipose fin ($r_s=-0.76$, $p=0.0009$), and (*b*) total number of red spots on both the body sides ($r_s=-0.52$, $p=0.048$). The lines give the regressions.

differed in the viability of their embryos (permutation test, male effect on embryo mortality: $p=0.002$; table 1).

All red coloration measures seemed to be independent of male size and age (the sires' overall redness, the number of red spots or red hue and saturation versus age or size measures: $-0.39<r_s<0.11$, $n=15$, always $p>0.05$). Embryo viability was negatively correlated with the sires' overall redness and the number of red spots (figure 2). Embryo survival increased with increasing dark pigmentation of their fathers as revealed by low modal grey values (figure 3). Increasing dark pigmentation was also linked to male age ($r_s=-0.53$, $p=0.04$) and male weight ($r_s=-0.51$, $p=0.05$), but embryo survival could not significantly be predicted by male age or size ($0.002<r_s<0.18$, always $p>0.05$).

Out of 1261 hatchlings that were released into the wild, 10 juveniles (0.8%) could be caught back 20 months later. They were sired by eight males that did not differ significantly from the other seven males in age or in any of the colour or size measurements (Kruskal–Wallis, always $p>0.05$). There was also no significant correlation between juveniles' size or condition factor and the sires' age, size or colour traits (Spearman's rank-order correlations, always $p>0.05$). The low recapture rate remains unexplained but could be linked to an extraordinary high water that occurred few months before recapture and that caused strong currents at the study site.

Spottiness, i.e. the total number of black, brown and red spots on both body sides, appeared to be heritable (figure 4*a*), while no evidence for a heritability of other

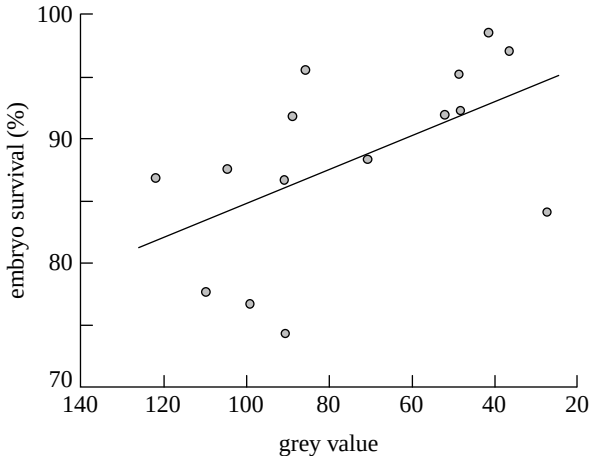


Figure 3. Modal grey value (most frequently occurring grey value, average of two body sides) versus embryo survival (regression line; $r_s=-0.60$, $p=0.018$). Low modal grey values indicate dark overall pigmentation.

pigmentation traits was found (correlation between sire and offspring, overall dark pigmentation: $r=-0.28$, $p>0.05$; overall redness: $r=-0.23$, $n=8$, $p>0.05$; red spots, figure 4*b*). We found no variation in the hue value of the red colour patches among the juveniles that could be recaptured.

4. DISCUSSION

In fishes, nuptial coloration can function both in inter- and intrasexual selection (Kodric-Brown 1998). In the case of the brown trout, the relative importance of agonistic

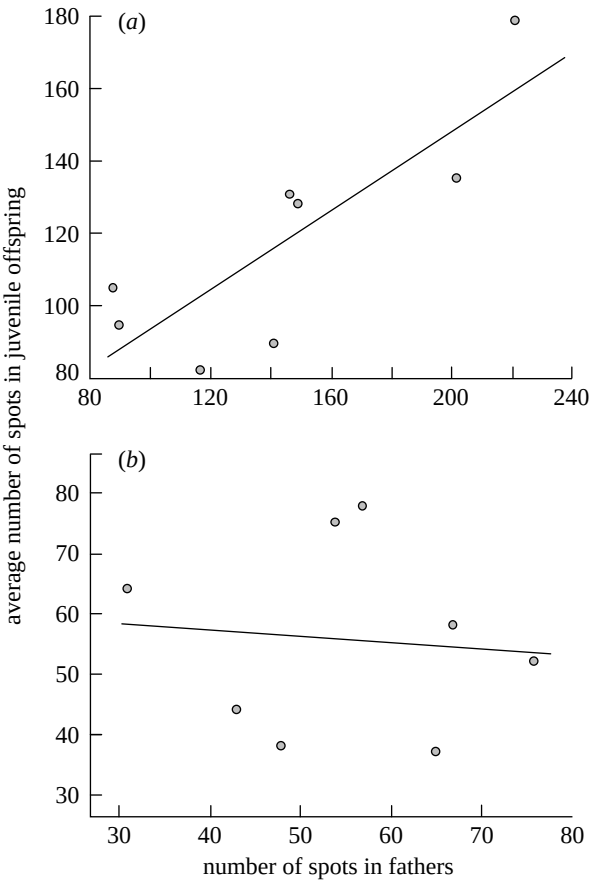


Figure 4. Regression of sires' spottiness versus average spottiness of their juvenile offspring. Total number of (*a*) spots on both body sides ($r=0.83$, $p=0.01$) and (*b*) red spots only ($r=-0.09$, $p>0.05$).

interactions between males and courtship to females is still unclear. It is even unclear whether there is any female mate choice in the brown trout (Petersson *et al.* 1999; Jacob *et al.* 2007). However, generalizations about a species' breeding system are difficult to draw because fishes often respond opportunistically to temporal or spatial variation in sex ratio, population density, habitat structure and other ecological factors (Kodric-Brown 1998). Our results suggest that if females choose and base their mate choice on colours, they should prefer darker males in order to increase the viability of their embryos. Contrary to what could be found in many other species (see §1 and below), they should not prefer red males. In fact, they should even avoid mating with bright and red males owing to the low viability of the embryos these males sire. However, we could not detect any positive or negative links between sire colour characteristics and the viability of their juvenile offspring. This could be due to either low statistical power or the different nature of embryo and juvenile mortality (Heath *et al.* 1999).

In salmonids, females are usually restricted in their choice of mates. They often defend spawning territories where they spawn their eggs in several batches, and males compete for access to these females (Fleming & Reynolds 2004; Quinn 2005). Large males are more successful in such interactions, and females seem to prefer larger males as they deposit more eggs with larger males (Foote 1989), and they are more aggressive towards small males than towards large males (Allen *et al.* 2007) or may delay spawning when courted by non-preferred males (Esteve 2005). Some *Oncorhynchus* species, especially the anadromous and non-anadromous forms of *Oncorhynchus nerka* (sockeye salmon and kokanee, respectively) or *Oncorhynchus mykiss* (steelhead and rainbow trout, respectively), develop intense red colours during the spawning season. These colours are carotenoid based (Bjerkeng *et al.* 1992; Craig *et al.* 2005), and, as in the three-spined stickleback (see §1), the red breeding colour of *O. nerka* has been found to positively influence mate choice (Craig & Foote 2001; Foote *et al.* 2004).

Animals often announce their aggressive stage and their fighting ability in colours and other characteristics (Huntingford & Turner 1987; Roulin 2004*b*), and fighting ability could be correlated with overall health and vigour and hence with good genes. However, it is not yet clear whether darker skin colours signal dominance or subordination in brown trout. Among juvenile fishes, social subordination has been linked to darker skin colours in rainbow trout (*O. mykiss*; Abbott *et al.* 1985), Atlantic salmon (*Salmo salar*; O'Connor *et al.* 1999) and Arctic charr (*S. alpinus*; Hoglund *et al.* 2000). Among spawners, however, the situation could be very different (Fleming & Reynolds 2004; Esteve 2005). A change to dark colours that may signal dominance or attractiveness during the spawning season is often seen in freshwater fishes (Kodric-Brown 1998).

There are different types of fish chromatophores, including melanophores that contain melanized organelles and that produce dark colours on the skin (Fujii 1993; Sugimoto 2002). Some chromatophores show very motile responses, for example, in response to their surroundings (some fishes are dark on a black background and pale on a white background; Sugimoto 2002). The variation in colours that we observed is, however, unlikely to reflect responses of the fish to their light environment. We controlled for such environmental variation by keeping the males for 14 days in one large unstructured tank before they were photographed.

Carotenoid-based colours can have a genetic basis (Craig *et al.* 2005), but the within-population variation is often due to diet and factors that influence carotenoid uptake and storage (Craig *et al.* 2005; Hadfield & Owens 2006). Darker skin patterns are usually due to higher melanin content (Hoglund *et al.* 2000; Pavlidis *et al.* 2006). Compared with carotenoid-based colours, melanin-based colours seem to have a stronger genetic basis (Hudon 1994; Hadfield & Owens 2006), but environmental influences are nevertheless possible (Hoglund *et al.* 2000; Fargallo *et al.* 2007). We found a significant father–offspring correlation in the total number of spots, i.e. this aspect of the skin coloration appears to be heritable. An analogous heritability has been observed in rainbow trout (Kause *et al.* 2003). We found no significant evidence for any heritability of red colour traits, but since our heritability analyses are based on a rather small sample size, such non-significant findings cannot be taken as evidence for a lack of heritability.

In a recent study on brown trout, Forsberg *et al.* (2007, p. 1863) found '… no apparent reproductive skew that would have indicated a strong 'good genes' effect'. However, they also found that their most successful males had a higher 'microsatellite score', i.e. these males were more different in their average genetics from the rest of the sample than males with lower reproductive success.

It remains unclear whether this microsatellite score is linked to variation in genetic load or to the origin of the breeders (the authors used a mixture of hatchery- and wild-born males). In the former case, within-population variation in mating success may be caused by variation in genetic load. Our findings would then suggest that the genetic load is revealed by melanin-based colour traits. However, contrary to other species, a mate preference for red colours would not promote indirect genetic benefits in the case of the brown trout.

The study was done with permission of the *Fischerei- und Jagdverwaltung des Kanton Luzern* and conforms to Swiss laws.

We thank P. Amrein for providing the fish; P. Amrein, B. von Siebenthal, D. Urbach and K. von Wattenwyl for their discussion and assistance in the field; and S. Cotton, A. Roulin and the anonymous reviewers for their constructive comments on the manuscript. The project was supported by the Swiss National Science Foundation.

REFERENCES

Abbott, J. C., Dunbrack, R. L. & Orr, C. D. 1985 The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). *Behaviour* **92**, 241–253.

Allen, C. S., Rich Jr, H. B. & Quinn, T. P. 2007 Condition-dependent reproductive tactics by large and small anadromous male sockey salmon *Oncorhynchus nerka*. *J. Fish Biol.* **70**, 1302–1307. (doi:10.1111/j.1095-8649.2007.01391.x)

Badyaev, A. V. & Young, R. L. 2004 Complexity and integration in sexual ornamentation: an example with carotenoid and melanin plumage pigmentation. *J. Evol. Biol.* **17**, 1317–1327. (doi:10.1111/j.1420-9101.2004.00781.x)

Bakker, T. C. M. & Mundwiler, B. 1994 Female mate choice and male red coloration in a natural 3-spined stickleback (*Gasterosteus aculeatus*) population. *Behav. Ecol.* **5**, 74–80. (doi:10.1093/beheco/5.1.74)

Bakker, T. C. M., Mazzi, D. & Zala, S. 1997 Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology* **78**, 1098–1104.

Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J., Mullen, W. & Huntingford, F. A. 2000 Carotenoid-based sexual coloration and body condition in nesting male sticklebacks. *J. Fish Biol.* **57**, 777–790. (doi:10.1111/j.1095-8649.2000.tb00274.x)

Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J. & Huntingford, F. A. 2001 Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. B* **268**, 71–76. (doi:10.1098/rspb.2000.1331)

Bates, D. M. 2005 Fitting linear mixed models in R. *R News* **5**, 27–30.

Bize, P., Gasparini, J., Klopfenstein, A., Altwegg, R. & Roulin, A. 2006 Melanin-based coloration is a nondirectionally selected sex-specific signal of offspring development in the Alpine swift. *Evolution* **60**, 2370–2380.

Bjerkeng, B., Storebakken, T. & Liaaenjensen, S. 1992 Pigmentation of rainbow trout from start feeding to sexual maturation. *Aquaculture* **108**, 333–346. (doi:10.1016/0044-8486(92)90117-4)

Candolin, U. 2000 Changes in expression and honesty of sexual signalling over the reproductive lifetime of sticklebacks. *Proc. R. Soc. B* **267**, 2425–2430. (doi:10.1098/rspb.2000.1301)

Craig, J. K. & Foote, C. J. 2001 Countergradient variation and secondary sexual color: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* **55**, 380–391. (doi:10.1111/j.0014-3820.2001.tb01301.x)

Craig, J. K., Foote, C. J. & Wood, C. C. 2005 Countergradient variation in carotenoid use between sympatric morphs of sockeye salmon (*Oncorhynchus nerka*) exposes nonanadromous hybrids in the wild by their mismatched spawning colour. *Biol. J. Linn. Soc.* **84**, 287–305. (doi:10.1111/j.1095-8312.2005.00430.x)

Esteve, M. 2005 Observations of spawning behaviour in Salmoninae: *Salmo*, *Oncorhynchus* and *Salvelinus*. *Rev. Fish Biol. Fish.* **15**, 1–21. (doi:10.1007/s11160-005-7434-7)

Evanno, G., Müller, R. & Wedekind, C. Submitted. Differential influence of maternal and paternal effects on embryo and juvenile survival in a salmonid.

Fargallo, J. A., Laaksonen, T., Korpimaki, E. & Wakamatsu, K. 2007 A melanin-based trait reflects environmental growth conditions of nestling male Eurasian kestrels. *Evol. Ecol.* **21**, 157–171. (doi:10.1007/s10682-006-0020-1)

Fleming, I. A. & Reynolds, J. D. 2004 Salmonid breeding system. In *Evolution illuminated: salmon and their relatives* (eds A. P. Hendry & S. C. Stearns), pp. 264–294. Oxford, UK: Oxford University Press.

Foote, C. J. 1989 Female mate preference in Pacific salmon. *Anim. Behav.* **38**, 721–723. (doi:10.1016/S0003-3472(89)80022-3)

Foote, C. J., Brown, G. S. & Hawryshyn, C. W. 2004 Female colour and male choice in sockeye salmon: implications for the phenotypic convergence of anadromous and nonanadromous morphs. *Anim. Behav.* **67**, 69–83. (doi:10.1016/j.anbehav.2003.02.004)

Forsberg, L. A., Dannewitz, J., Petersson, E. & Grahn, M. 2007 Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout—female fishing for optimal MHC dissimilarity. *J. Evol. Biol.* **20**, 1859–1869. (doi:10.1111/j.1420-9101.2007.01380.x)

Frischknecht, M. 1993 The breeding coloration of male 3-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigor. *Evol. Ecol.* **7**, 439–450. (doi:10.1007/BF01237640)

Fujii, R. 1993 Coloration and chromatophores. In *The physiology of fishes* (ed. D. H. Evans), pp. 535–562. Boca Raton, FL: CRC Press.

Grafen, A. 1990 Biological signals as handicaps. *J. Theor. Biol.* **144**, 517–546.

Grether, G. F. 2000 Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**, 1712–1724. (doi:10.1111/j.0014-3820.2000.tb00715.x)

Grether, G. F., Kasahara, S., Kolluru, G. R. & Cooper, E. L. 2004 Sex-specific effects of carotenoid intake on the immunological response to allografts in guppies (*Poecilia reticulata*). *Proc. R. Soc. B* **271**, 45–49. (doi:10.1098/rspb.2003.2526)

Grether, G. F., Kolluru, G. R., Rodd, F. H., de la Cerda, J. & Shimazaki, K. 2005 Carotenoid availability affects the development of a colour-based mate preference and the sensory bias to which it is genetically linked. *Proc. R. Soc. B* **272**, 2181–2188. (doi:10.1098/rspb.2005.3197)

Griffith, S. C., Parker, T. H. & Olson, V. A. 2006 Melanin- versus carotenoid-based sexual signals: is the difference really so black and red? *Anim. Behav.* **71**, 749–763. (doi:10.1016/j.anbehav.2005.07.016)

Hadfield, J. D. & Owens, I. P. F. 2006 Strong environmental determination of a carotenoid-based plumage trait is not mediated by carotenoid availability. *J. Evol. Biol.* **19**, 1104–1114. (doi:10.1111/j.1420-9101.2006.01095.x)

Heath, D. D., Fox, C. W. & Heath, J. W. 1999 Maternal effects on offspring size: variation through early development of Chinook salmon. *Evolution* **53**, 1605–1611. (doi:10.2307/2640906)

Hoglund, E., Balm, P. H. M. & Winberg, S. 2000 Skin darkening, a potential social signal in subordinate Arctic charr (*Salvelinus alpinus*): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. *J. Exp. Biol.* **203**, 1711–1721.

Houde, A. E. 1997 *Sex, color and mate choice in guppies*. Princeton, NJ: Princeton University Press.

Hudon, J. 1994 Biotechnological applications of research on animal pigmentation. *Biotechnol. Adv.* **12**, 49–69. (doi:10.1016/0734-9750(94)90290-9)

Huntingford, F. A. & Turner, A. M. 1987 *Animal conflict*. London, UK: Chapman & Hall.

Jacob, A., Nusslé, S., Britschgi, A., Müller, R. & Wedekind, C. 2007 Male dominance linked to size and age, but not to 'genetic quality' in brown trout (*Salmo trutta*). *BMC Evol. Biol.* **71**, 207. (doi:10.1186/1471-2148-7-207)

Kause, A., Ritola, O., Paananen, T., Eskelinen, U. & Mantysaari, E. 2003 Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. *J. Fish Biol.* **62**, 610–622. (doi:10.1046/j.1095-8649.2003.00051.x)

Kodric-Brown, A. 1998 Sexual dichromatism and temporary color changes in the reproduction of fishes. *Am. Zool.* **38**, 70–81. (doi:10.1093/icb/38.1.70)

Kodric-Brown, A. & Nicoletto, P. F. 2001 Age and experience affect female choice in the guppy (*Poecilia reticulata*). *Am. Nat.* **157**, 316–323. (doi:10.1086/319191)

Kolluru, G. R., Grether, G. F., South, S. H., Dunlop, E., Cardinali, A., Liu, L. & Carapiet, A. 2006 The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*). *Biol. J. Linn. Soc.* **89**, 301–309. (doi:10.1111/j.1095-8312.2006.00675.x)

Krinsky, N. I. 1993 Actions of carotenoids in biological systems. *Annu. Rev. Nutr.* **13**, 561–587. (doi:10.1146/annurev.nu.13.070193.003021)

Majerus, M. E. N. & Mundy, N. I. 2003 Mammalian melanism: natural selection in black and white. *Trends Genet.* **19**, 585–588. (doi:10.1016/j.tig.2003.09.003)

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x)

Masvaer, M., Liljedal, S. & Folstad, I. 2004 Are secondary sex traits, parasites and immunity related to variation in primary sex traits in the Arctic charr? *Proc. R. Soc. B* **271**, S40–S42. (doi:10.1098/rsbl.2003.0090)

McGraw, K. J. 2005 The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Anim. Behav.* **69**, 757–764. (doi:10.1016/j.anbehav.2004.06.022)

McGraw, K. J. 2007 Dietary mineral content influences the expression of melanin-based ornamental coloration. *Behav. Ecol.* **18**, 137–142. (doi:10.1093/beheco/arl059)

McGraw, K. J. & Ardia, D. R. 2003 Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *Am. Nat.* **162**, 704–712. (doi:10.1086/378904)

Milinski, M. & Bakker, T. C. M. 1990 Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**, 330–333. (doi:10.1038/344330a0)

Neff, B. D. & Pitcher, T. E. 2005 Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol. Ecol.* **14**, 19–38. (doi:10.1111/j.1365-294X.2004.02395.x)

Niecke, M., Rothlaender, S. & Roulin, A. 2003 Why do melanin ornaments signal individual quality? Insights from metal element analysis of barn owl feathers. *Oecologia* **137**, 153–158. (doi:10.1007/s00442-003-1307-3)

O'Connor, K. I., Metcalfe, N. B. & Taylor, A. C. 1999 Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*? *Anim. Behav.* **58**, 1269–1276. (doi:10.1006/anbe.1999.1260)

Olson, V. A. & Owens, I. P. F. 1998 Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510–514. (doi:10.1016/S0169-5347(98)01484-0)

Parker, T. H. 2003 Genetic benefits of mate choice separated from differential maternal investment in red junglefowl (*Gallus gallus*). *Evolution* **57**, 2157–2165. (doi:10.1111/j.0014-3820.2003.tb00393.x)

Pavlidis, M., Papandroulakis, N. & Divanach, P. 2006 A method for the comparison of chromaticity parameters in fish skin: preliminary results for coloration pattern of red skin *Sparidae*. *Aquaculture* **258**, 211–219. (doi:10.1016/j.aquaculture.2006.05.028)

Peters, A. 2007 Testosterone and carotenoids: an integrated view of trade-offs between immunity and sexual signalling. *Bioessays* **29**, 427–430. (doi:10.1002/bies.20563)

Petersson, E., Järvi, T., Olsen, H., Mayer, I. & Hedenskog, M. 1999 Male–male competition and female choice in brown trout. *Anim. Behav.* **57**, 777–783. (doi:10.1006/anbe.1998.1043)

Pitcher, T. E. & Neff, B. D. 2006 MHC class IIB alleles contribute to both additive and nonadditive genetic effects on survival in Chinook salmon. *Mol. Ecol.* **15**, 2357–2365. (doi:10.1111/j.1365-294X.2006.02942.x)

Quinn, T. P. 2005 *The behavior and ecology of Pacific salmon and trout*. Seattle, WA: University of Washington Press.

Rifflart, R., Marchand, F., Rivot, E. & Bagliniere, J. L. 2006 Scale reading validation for estimating age from tagged fish recapture in a brown trout (*Salmo trutta*) population. *Fish. Res.* **78**, 380–384. (doi:10.1016/j.fishres.2005.11.018)

Roulin, A. 2004*a* Proximate basis of the covariation between a melanin-based female ornament and offspring quality. *Oecologia* **140**, 668–675. (doi:10.1007/s00442-004-1636-x)

Roulin, A. 2004*b* The evolution, maintenance and adaptive function of genetic colour polymorphism in bird. *Biol. Rev.* **79**, 1–34. (doi:10.1017/S1464793104006487)

Roulin, A. 2007 Melanin pigmentation negatively correlates with plumage preening effort in barn owls. *Funct. Ecol.* **21**, 264–271. (doi:10.1111/j.1365-2435.2006.01229.x)

Roulin, A., Dauwe, T., Blust, R., Eens, M. & Beaud, M. 2006 A link between eumelanism and calcium physiology in the barn owl. *Naturwissenschaften* **93**, 426–430. (doi:10.1007/s00114-006-0128-8)

Rudolfsen, G., Figenschou, L., Folstad, I., Nordeide, J. T. & Soreng, E. 2005 Potential fitness benefits from mate selection in the Atlantic cod (*Gadus morhua*). *J. Evol. Biol.* **18**, 172–179. (doi:10.1111/j.1420-9101.2004.00778.x)

Rudolfsen, G., Figenschou, L., Folstad, I., Tveiten, H. & Figenschou, M. 2006 Rapid adjustments of sperm characteristics in relation to social status. *Proc. R. Soc. B* **273**, 325–332. (doi:10.1098/rspb.2005.3305)

Skarstein, F. & Folstad, I. 1996 Sexual dichromatism and the immunocompetence handicap: an observational approach using Arctic charr. *Oikos* **76**, 359–367. (doi:10.2307/3546208)

Skarstein, F., Folstad, I. & Ronning, H. P. 2005 Spawning colouration, parasites and habitat selection in *Salvelinus alpinus*: initiating speciation by sexual selection? *J. Fish Biol.* **67**, 969–980. (doi:10.1111/j.0022-1112.2005.00796.x)

Smith, C., Barber, I., Wootton, R. J. & Chittka, L. 2004 A receiver bias in the origin of three-spined stickleback mate choice. *Proc. R. Soc. B* **271**, 949–955. (doi:10.1098/rspb.2004.2690)

Steven, D. M. 1948 Studies on animal carotenoids. 1. Carotenoids of the brown trout (*Salmo trutta* Linnaeus). *J. Exp. Biol.* **25**, 369–387.

Stevens, M., Parraga, C. A., Cuthill, I. C., Partridge, J. C. & Troscianko, T. S. 2007 Using digital photography to study animal coloration. *Biol. J. Linn. Soc.* **90**, 211–237. (doi:10.1111/j.1095-8312.2007.00725.x)

Sugimoto, M. 2002 Morphological color changes in fish: regulation of pigment cell density and morphology. *Microsc. Res. Tech.* **58**, 496–503. (doi:10.1002/jemt.10168)

Wedekind, C. & Müller, R. 2004 The experimental rearing of large salmonid eggs in Petri dishes. *Funct. Ecol.* **18**, 138–140. (doi:10.1111/j.1365-2435.2004.00822.x)

Wedekind, C., Meyer, P., Frischknecht, M., Niggli, U. A. & Pfander, H. 1998 Different carotenoids and potential information content of red coloration of male three-spined stickleback. *J. Chem. Ecol.* **24**, 787–801. (doi:10.1023/A:1022365315836)

Wedekind, C., Müller, R. & Spicher, H. 2001 Potential genetic benefits of mate selection in whitefish. *J. Evol. Biol.* **14**, 980–986. (doi:10.1046/j.1420-9101.2001.00349.x)

Wedekind, C., Walker, M., Portmann, J., Cenni, B., Müller, R. & Binz, T. 2004 MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. *J. Evol. Biol.* **17**, 11–18. (doi:10.1046/j.1420-9101.2004.00669.x)

Welch, A. M., Semlitsch, R. D. & Gerhardt, H. C. 1998 Call duration as an indicator of genetic quality in male gray tree frogs. *Science* **280**, 1928–1930. (doi:10.1126/science.280.5371.1928)

Appendix II - Publications in the context of fisheries

In collaboration with the Study Group of Fisheries-Induced Adaptive Change (SGFIAC) established by The International Council for Exploration of the Sea (ICES), under the direction of Mikko Heino and Ulf Dieckmann.

Evolutionary impact assessment: Accounting for evolutionary consequences of fishing in an ecosystem approach to fisheries management

Ane T. Laugen, Georg H. Engelhard, Rebecca Whitlock, Robert Arlinghaus, Dorothy Dankel, Erin S. Dunlop, Anne Maria Eikeset, Katja Enberg, Christian Jørgensen, Shuichi Matsumura, Sébastien Nusslé, Davnah Urbach, Loïc Baulier, David S. Boukal, Bruno Ernande, Fiona Johnston, Fabian Mollet, Heidi Pardoe, Nina O. Therkildsen, Silva Uusi-Heikkila, Anssi Vainikka, Mikko Heino, Adriaan D. Rijnsdorp, and Ulf Dieckmann

Manuscript

Can fisheries-induced evolution shift reference points for fisheries management?

Mikko Heino, Loïc Baulier, David S. Boukal, Bruno Ernande, Fiona D. Johnston, Fabian Mollet, Heidi Pardoe, Nina O. Therkildsen, Silva Uusi-Heikkilä, Anssi Vainikka, Robert Arlinghaus, Dorothy J. Dankel, Erin S. Dunlop, Anne Maria Eikeset, Katja Enberg, Georg H. Engelhard, Christian Jørgensen, Ane T. Laugen, Shuichi Matsumura, Sébastien Nusslé, Davnah Urbach, Rebecca Whitlock, Adriaan D. Rijnsdorp, and Ulf Dieckmann

Manuscript in prep for ICES Journal of Marine Science

Evolutionary impact assessment: Accounting for evolutionary consequences of fishing in an ecosystem approach to fisheries management

Evolutionary impact assessment: A framework for managing evolving resources

Abstract

Human activity can cause rapid evolutionary changes, but quantifying such change can be difficult. For example, while several commercial fish stocks have undergone remarkable phenotypic changes within just a few generations, the extent to which this reflects evolutionary change rather than phenotypic plasticity is the subject of debate. However, increasing evidence supports the premise that modern fisheries exert strong directional selection on the life history, behavior, physiology, and morphology of exploited fish. A particular concern about fisheries-induced evolution is that it can be much more difficult or slow to reverse than demographic or phenotypically plastic changes. Potential fisheries-induced evolution may change the utility of fish stocks, which in turn can modify the ecological services through which living aquatic resources provide value to society. For these reasons, quantifying the evolutionary effects of fishing is important for both economic and ecological reasons. Such assessment may assist discussions among stakeholders and facilitate the management of affected stocks. Here we describe evolutionary impact assessment (EvoIA) as a structured approach for assessing the evolutionary consequences of fishing and for evaluating the merits of alternative management options. EvoIA will (i) contribute to the ecosystem approach to fisheries management by clarifying how evolution alters stock properties and ecological relations, (ii) support the precautionary approach to fisheries management by addressing a previously overlooked source of uncertainty and risk, and (iii) help realize the Johannesburg summit's commitment to the restoration of sustainable fisheries.

This article has been prepared jointly by participants of the 2008 Annual Meeting of the Study Group on Fisheries-Induced Adaptive Change (SGFIAC) of the International Council for the Exploration of the Sea (ICES). ATL, GHE, RW, and UD coordinated preparations and integrated the writing. RA, DD, UD, ESD, AME, KE, GHE, CJ, ATL, SM, SN, DU, and RW wrote and reviewed sections. LB, DSB, BE, MH, FJ, FM, HP, ADR, NOT, SUH, and AV contributed suggestions and comments. In 2009, SGFIAC became the Working Group on Fisheries-induced Evolution (WGEVO). For further information about the working group please contact the WGEVO chairs UD, MH, or ADR.

Can fisheries-induced evolution shift reference points for fisheries management?

Abstract

Biological reference points are important tools for fisheries management. Reference points are not static, but may be altered when a population's environment or the population itself changes. Fisheries-induced evolution is one mechanism that can alter population characteristics, leading to "shifting" reference points. This occurs through two pathways: by modifying the underlying biological processes, and by leading to changes in the perception of a system. The former implies that "true" reference points are changing, whereas the latter implies that the yardstick used to quantify a system's status is changing. Unaccounted-for shifting of either kind means that reference points gradually lose their intended meaning. This can lead to increased precaution, which is safe but potentially costly. Shifts can also occur in more perilous directions, such that actual risks are greater than anticipated. Our qualitative analysis suggests that all commonly used reference points are susceptible to shifting under fisheries-induced evolution, including the widely-used limit and precautionary reference points for spawning-stock biomass (B_{lim} and B_{pa}) and fishing mortality (F_{lim} and F_{pa}). Our findings call for increased awareness of fisheries-induced changes and highlight the value of basing reference points on adequately updated information, to capture changes in the biological processes that drive fish population dynamics.

This article has been prepared jointly by participants of the 2008 Annual Meeting of the Study Group on Fisheries-Induced Adaptive Change (SGFIAC) of the International Council for the Exploration of the Sea (ICES). MH and UD coordinated preparations and integrated the writing. LB, DSB, UD, BE, MH, FJ, FM, HP, ADR, NOT, SUH, and AV wrote and reviewed sections. RA, DJD, ESD, AME, KE, GHE, CJ, ATL, SM, SN, DU, and RW contributed suggestions and comments. In 2009, SGFIAC became the Working Group on Fisheries-induced Evolution (WGEVO). For further information about the working group please contact the WGEVO chairs UD, MH, or ADR.

Appendix III - The relative influence of body mass, metabolic rate and sperm competition on the spermatogenic cycle length in mammals

Cycle length of spermatogenesis in shrews (Mammalia: Soricidae) with high and low metabolic rates and different mating systems

Roumen Parapanov, Sébastien Nusslé, and Peter Vogel

Published in Biology of Reproduction

Relationships of basal metabolic rate, relative testis size and cycle length of spermatogenesis in shrews (Mammalia, Soricidae)

Roumen Parapanov, Sébastien Nusslé, Jacques Hausser, and Peter Vogel

Published in Animal Reproduction Science

Histological description of seminiferous epithelium and cycle length of spermatogenesis in the water shrew Neomys fodiens (Mammalia: Soricidae)

Roumen Parapanov, Sébastien Nusslé, Jacques Hausser, and Peter Vogel

Published in Reproduction, Fertility & Development

Testis size, sperm characteristics and testosterone concentrations in four species of shrews (Mammalia, Soricidae)

Roumen Parapanov, Sébastien Nusslé, Michel Crausaz, Alfred Senn, Jacques Hausser, and Peter Vogel

Published in Animal Reproduction Science

The relative influence of body mass, metabolic rate and sperm competition on the spermatogenic cycle length in mammals

Roumen Parapanov, Nicolas Salamin, Sébastien Nusslé, Jacques Hausser, and Peter Vogel

Submitted to BMC Evolutionary Biology

BIOLOGY OF REPRODUCTION **76,** 833–840 (2007)
Published online before print 31 January 2007.
DOI 10.1095/biolreprod.106.058073

Cycle Length of Spermatogenesis in Shrews (Mammalia: Soricidae) with High and Low Metabolic Rates and Different Mating Systems[1]

Roumen Parapanov,[2] Sébastien Nusslé, and Peter Vogel

Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland

ABSTRACT

The aim of the present study was to establish and compare the durations of the seminiferous epithelium cycles of the common shrew *Sorex araneus*, which is characterized by a high metabolic rate and multiple paternity, and the greater white-toothed shrew *Crocidura russula*, which is characterized by a low metabolic rate and a monogamous mating system. Twelve *S. araneus* males and fifteen *C. russula* males were injected intraperitoneally with 5-bromodeoxyuridine, and the testes were collected. For cycle length determinations, we applied the classical method of estimation and linear regression as a new method. With regard to variance, and even with a relatively small sample size, the new method seems to be more precise. In addition, the regression method allows the inference of information for every animal tested, enabling comparisons of different factors with cycle lengths. Our results show that not only increased testis size leads to increased sperm production, but it also reduces the duration of spermatogenesis. The calculated cycle lengths were 8.35 days for *S. araneus* and 12.12 days for *C. russula*. The data obtained in the present study provide the basis for future investigations into the effects of metabolic rate and mating systems on the speed of spermatogenesis.

male reproductive tract, spermatogenesis, testis

INTRODUCTION

Spermatogenesis is the process whereby the spermatogonial stem cells on the basal membrane of the seminiferous tubules divide and differentiate, giving rise to testicular spermatozoa at the luminal surface. During this process, the germ cells are arranged into cellular associations and are divided into named stages. These stages proceed in succession over time in any given region of the tubule, and this process is defined as the seminiferous epithelium cycle [1]. The types of germ cell involved in this process are similar in all mammals [2, 3]. Differences between species exist with regard to the number of germ cells, the duration of spermatogenesis, and the topographical arrangement of the spermatogenetic stages [4].

The duration of the cycle has been studied in many species. It seems to be rather constant within species but varies between species. Within rodents, the cycle length may be as short as 6.7 days, as in the bank vole *Clethrionomys glareolus* [5], and up to 17 days, as in the Chinese hamster *Cricetulus griseus* [6]. In primates, the cycle length is 9.4 days in the cynomolgus monkey *Macaca fascicularis* [7] and 16 days in humans [8]. Within marsupials, the cycle duration is rather long, from 15.7 days in *Trichosurus vulpecula* [9] to 17.3 days in *Didelphis albiventris* [10]. There is no obvious factor to explain the differences between species.

Body size and the related energy metabolism appear to be important factors in determining the physiological rates [11–13]. However, a possible influence on spermatogenesis has never been tested. According to McNab [14], a higher metabolic rate may increase cell cycle speed and tissue synthesis. Therefore, we conclude that metabolic rate is a potential factor in spermatogenesis. In the context of energy metabolic rate, shrews represent an interesting model. The two recognized shrew subfamilies, Soricinae (red-toothed shrews) and Crocidurinae (white-toothed shrews), show important differences. The Soricinae (e.g., *Sorex araneus*) are known for their extremely high metabolic rates, which are 200–300% of the value expected for their body sizes [15–18]. In contrast, the Crocidurinae generally have metabolic rates just above the expected value (about 120%), with some desert species going as low as 75% of the expected value [19]. Therefore, we studied the duration of the spermatogenesis cycle in shrews.

To the best of our knowledge, this type of study has not been reported previously, with the exception of histological descriptions of the stages that characterize the cycle of spermatogenesis. According to Plöen et al. [20] and Garagna et al. [21], in Soricinae, the seminiferous tubules show a segmental arrangement, with usually just one stage found per tubule cross-section. This topographical arrangement is present in the majority of mammalian species. The situation is similar in the Crocidurinae, as shown for the Japanese *Crocidura watasei* [22] and the Asian *Suncus murinus* [23]. In these studies, the authors subdivided the cycle of the seminiferous epithelium according to the morphological characteristics of spermatids, with particular emphasis on their nuclei and acrosomic system [24]. Based on this method, the number of stages of the cycle varies between species, with 10 stages in *S. araneus* [21], 12 stages in *C. watasei* [22], and 13 stages in *S. murinus* [23]. However, the duration of each stage has yet to be determined.

In recent decades, the presence or absence of sperm competition has been shown to be an important factor with influence on sperm production [25]. When sperm competition occurs, males are often selected on the basis of having an increased quantity of sperm. This is evidenced by a significant correlation between the strength of competition and testis size [26–29]. Larger testis volume is linked to higher total sperm production. In this context, shrews are again an interesting model. In multiple copulators, such as *S. araneus*, sperm competition seems to occur, and a litter may have up to five fathers [30–32]. In contrast, within the Crocidurinae, which is the most extensively studied species, *Crocidura russula* seems to be rather monogamous [33] and multiple paternity has never been demonstrated [34]. Besançon [35] has reported rather small testes for *C. russula* compared with the large testes of *S. araneus*. If increased sperm production is linked to an increase

[1]Supported by the University of Lausanne, Switzerland.
[2]Correspondence: FAX: 41 21 692 4165;
e-mail: Roumen.Parapanov@unil.ch

Received: 12 October 2006.
First decision: 13 November 2006.
Accepted: 29 January 2007.

ISSN: 0006-3363. http://www.biolreprod.org

in testis size, sperm competition may also be expected to increase sperm production per unit time and testis volume. As shown previously, both an increase in metabolic rate (for any reason) and sperm competition may lead to a higher rate of sperm production. The present study examines the duration of the sperm production cycle in two species with very different life history strategies [36, 37], which should establish a standard for further comparisons.

In the present study, we determined the duration of the cycle of spermatogenesis in both the common shrew *S. araneus* and the greater white-toothed shrew *C. russula*. The dynamics of sperm production were determined by tracking 5-bromodeoxyuridine (BrdU) in the DNA of S-phase germ cells, since BrdU was incorporated into the nuclei of cells that were duplicating their DNAs in preparation for mitosis or meiosis.

MATERIALS AND METHODS

Animals

S. araneus is widely distributed in the northern Palaearctic region. From April to June, we captured twelve adult *S. araneus* (chromosomal race of Valais, now considered to be *S. antinorii* [38]), weighing 10.9 ± 0.7 g, in the regions of Trient and Grand St-Bernard in the Alps at an altitude of 1400–2200 m.

C. russula, which originated in Africa [39], is widespread in south-western Europe. Fifteen adult males weighing 14.2 ± 1.3 g were captured during the breading season (February to August) in the region of Lausanne, Switzerland (altitude 300 m). The shrews were kept in a special building with a roof that protected them from direct rain but with walls of wire mesh that exposed the animals to natural fluctuations in temperature and humidity according to the weather. The shrews were caged individually, on natural soil. They had ad libitum access to water and food, which was composed of minced meat with vitamin supplements and mealworms.

Administration of BrdU

BrdU (BD Biosciences, Pharmingen) was administered to each male as a single i.p. injection at a dose of 50 mg/kg. *S. araneus* males were treated in groups of three animals and killed by halothane overdose at 3 h, 8 days 3 h, 12 days 3 h or 16 days 3 h after injection of BrdU. The *C. russula* males were killed according to the following schedule: four males at 3 h, three at 8 days 3 h, three at 12 days 3 h, three at 16 days 3 h, and two at 24 days 3 h after the administration of BrdU. The testes were removed, weighed, fixed in a 4% solution of paraformaldehyde for 3 days, dehydrated in a graded series of ethanol, and embedded in Paraplast. Histological sections (3-µm thickness) of the testes were dewaxed and rehydrated. BrdU was localized using the Zymed BrdU Staining Kit (Invitrogen). Endogenous peroxidase was inactivated with 3% (v/v) H_2O_2 in methanol for 10 min at room temperature. To expose BrdU for immunohistochemical localization, DNA was denatured in 0.17% (v/v) trypsin solution for 10 min at 37°C in a humid chamber. Incubation with a biotinylated anti-BrdU antibody was performed for 1 h at room temperature. Sections were then incubated with streptavidin-HRP for 10 min at room temperature. DAB substrate solution was added to cover the tissue sections and they were incubated for 5 min or until color development. PAS-hematoxylin staining was performed for the identification of spermatogenic stages. Incubation with 1% periodic acid (for 10 min at room temperature) and staining with Schiff reagent (for 30 min at room temperature) was followed by incubation with Mayer hematoxylin solution (5 min at room temperature). Histological preparations were observed under an Axiophot microscope (Zeiss, Germany).

Stage Frequencies of the Seminiferous Epithelium Cycle

The ten stages (I–X) of the seminiferous epithelium cycle were determined following the criteria applied previously to *S. araneus* [20, 21]. In *C. russula*, we identified the 12 stages of the seminiferous epithelium according to the classification proposed by Adachi et al. [22] for *Crocidura watasei*. For direct comparisons with *S. araneus*, we pooled stages VIII-IX and X-XI of *Crocidura*, and renamed them stages VIII and IX respectively; thus, now stage XII corresponds to stage X in *S. araneus* (see Table 2).

The relative duration of each stage of the cycle in both species was determined for testes sections that were counterstained with PAS-hematoxylin. We counted only round cross-sections of the seminiferous tubules.

Stages of short duration should appear rarely, while stages of long duration should appear frequently. Therefore, the stage frequencies (expressed as percentages) correspond to the relative durations of the stages of the cycle. We counted 200–400 tubular cross-sections per testis. In all, 7000 tubules from 12 *S. araneus* shrews and 6000 tubules from 15 *C. russula* shrews were scored. Stage frequencies were calculated for all animals using the equation:

$$\text{Stage frequency}(\%) = \frac{\text{number of tubules of given stage}}{\text{number of all tubules}} \times 100 \qquad (1)$$

The mean value was calculated for the frequency of each stage.

BrdU Staining Frequency

The estimation of staining frequency was based on the percentage of tubules of a given stage that contained the most-advanced germ cells that were labeled with BrdU, as follows:

$$\text{Staining frequency}(\%) = \frac{\text{number of tubules containing the most advanced labeled cells in a given stage}}{\text{number of all tubules of that stage}} \times 100 \qquad (2)$$

We determined the staining frequency by scoring more than 60 tubule cross-sections for each animal.

Duration of the Seminiferous Epithelium Cycle

Two methods were used to estimate the cycle duration. The first method was that of Rosiepen et al. [40, 41]. Following this formula, the duration of the seminiferous epithelium cycle was calculated based on the stage frequencies and BrdU staining frequencies for two time-points. We used the time-point of 3 h after BrdU injection as a reference group. Between this time-point and the other time-points, we calculated the duration of one cycle of spermatogenesis. For example, in *S. araneus,* the duration was calculated as follows: at 3 h after BrdU injection, (reference group), the most-advanced labeled cells were at stage VI, while at 8 days 3 h after BrdU injection in animal #2, the most-advanced labeled cells were also in stage VI.

The time interval ΔT was calculated using the equation:

$$\Delta T = (\text{staining frequency of stage VI at 3 h} \times \text{stage frequency of stage VI}) - \left(\begin{array}{c}\text{staining frequency of stage VI at 8 days 3 h} \\ \times \text{stage frequency of stage VI}\end{array}\right)/100. \qquad (3)$$

In the second method, we estimated the duration of the seminiferous epithelium cycle by linear regression. The relationship between the relative time in percentage (Y) of the cycle (i.e., the percentage of the cycle performed) to the real time in hours (X) is described as a regression function of the equation:

$$Y = aX + b \qquad (4)$$

where Y is the relative time in percentage, X is real time in hours, a is the slope of the regression (the increase in relative time per unit real time), and b is the intercept (the percentage of the cycle that would have been stained at real time T = 0).

For example, in *S. araneus*, the most-advanced labeled cells at 3 h after BrdU injection were in stage VI. This corresponds to the sum of the frequencies of stages I to V plus the (staining frequency × stage frequency of stage VI). This is the relative time in percentage at 3 h. At 8 days 3 h (195 h real time), the most-advanced labeled cells were in the same stage. We added stages VII–V plus the (staining frequency × stage frequency of stage VI) at 8 days 3 h. In the same way, we calculated the relative time (%) for the other time-points of 12 days 3 h and 16 days 3 h after BrdU injection.

The duration of one cycle (100% relative time) was inferred from the regression function and is given as the value that was used for the comparisons. The standard deviation was also inferred from the linear regression.

Ethical Considerations

This project was performed under authorization number 1707.1 VD. The number of samples was kept as low as possible, since all species of shrew are protected animals in Switzerland. However, the loss of some males from a local population has no detrimental consequences due to the rather high population sizes of these two species. In the Lausanne population on the campus of the University, we have trapped and individually marked over 500 individuals of *C. russula* in 4 yr [34, 42].

TABLE 1. Comparative parameters in *Sorex araneus* and *Crocidura russula*.

Parameters	*Sorex araneus*	*Crocidura russula*
Body mass (g)	10.9 ± 0.7	14.2 ± 1.3
Testes mass (g)	0.173 ± 0.021	0.044 ± 0.004
Relative testis mass (%)	1.60 ± 0.21	0.31 ± 0.47
Spermatogenic cycle length (day)[a]	8.47 ± 0.26	12.42 ± 0.60
Spermatogenic cycle length (day)[b]	8.35 ± 0.13	12.12 ± 0.19
Total duration of spermatogenesis (day)[b]	~37.6	~54.7
Basal metabolic rate (BMR %)[c]	251 [16]	116 [19]
Multiple paternity	+[32]	−[33]

[a] Duration calculated according to method used by Rosiepen et al. [40, 41].
[b] Duration calculated by linear regression method.
[c] BMR % = BMR as a percentage of the value expected from the allometric relationship.

Statistical Analysis

All the data are presented as the mean ± SEM. Analysis of regression was performed using software R (R Development Core Team, 2006). The significance level was considered to be $P < 0.05$. To test the differences between *Sorex* and *Crocidura*, we applied the *t*-test after testing for normality.

RESULTS

Testicular Weights

During the period from the administration of BrdU to killing, we examined the general health of the treated animals. BrdU administration was well-tolerated and no negative effects were observed in either species. Measurements of body mass and testicular size are given in Table 1. The absolute testes mass of *S. araneus* (0.17 ± 0.02 g) was significantly higher than that of *C. russula* (0.040 ± 0.004 g) (Welsch modified *t*-test for non-equal variance; $t = -20.8759$, df = 11.481, $P < 0.0001$), and the relative testes size expressed as a percentage of body weight was also significantly higher in *S. araneus* than in *C. russula* (Welsch modified *t*-test for non-equal variance; $t = -20.5506$, df = 11.85, $P < 0.0001$).

BrdU Immunohistochemistry

In *S. araneus*, 3 h after BrdU injection, the label was localized in the nuclei of preleptotene spermatocytes at stage VI. This stage was used as a reference, and it contained the most-advanced labeled cells at 3 h (Fig. 1a). Stage VI began after spermiation and was characterized by the presence of round spermatids in the luminal compartment of the tubule. Residual bodies were found in the seminiferous epithelium. Two spermatocyte generations were present. The preleptotene spermatocytes were visible in the vicinity of the basement membrane, which was marked by BrdU. Pachytene spermatocytes were situated in the middle region of the seminiferous epithelium. At 8 days 3 h, the most-advanced cells that contained BrdU were pachytene spermatocytes in the same stage (Fig. 1b). At 12 days 3 h, the most-advanced BrdU-labeled cells were newly formed round spermatids at stage I (Fig. 1c). This stage began after the second meiotic division of the secondary spermatocytes and was characterized by the presence of two generations of spermatids: round spermatids in the middle region of the seminiferous epithelium and elongated spermatids in the luminal compartment. Type A spermatogonia overlying the basal membrane and small pachytene spermatocytes were also observed. At 16 days 3 h in two animals, the most-advanced BrdU-labeled cells were round spermatids at stage V (Fig. 1d) and at stage VI in one animal (Fig. 1e). Stage V was characterized by the presence of elongated spermatids that were ready to be released into the seminiferous lumen and round spermatids with an acrosome that covered half of the nucleus.

In *C. russula*, the most-advanced BrdU-labeled cells at 3 h, 12 days 3 h, and 24 days 3 h after BrdU injection were in stage VI (Fig. 2). In this stage, we observed cell associations similar to those seen for *S. araneus*. One generation of round spermatids and residual bodies were recognized in the seminiferous epithelium and two generations of spermatocytes (preleptotene and pachytene) were present in this stage. At 3 h, the most-advanced labeled cells were the preleptotene spermatocytes (Fig. 2a). At 12 days 3 h, BrdU was detected in pachytene spermatocytes (Fig. 2d), and at 24 days 3 h, the label was detected in round spermatids (Fig. 2f). At 8 days 3 h, the most-advanced labeled cells were pachytene spermatocytes at stage III in two animals (Fig. 2b) and at stage IV in one animal (Fig. 2c). In stage III, the acrosomal granule began to flatten on the surface of the spermatid nucleus. Maturing spermatids were also present at this stage. Stage IV was characterized by the beginning of the formation of the head caps on the nuclei of the spermatids. At 16 days 3 h after BrdU injection, the most-advanced labeled cells were newly formed round spermatids in stage I (Fig. 2e). This stage was characterized by the presence of two generations of spermatids: maturing spermatids and round spermatids that contained BrdU.

Stage Frequency, Staining Frequency, and Duration of the Seminiferous Epithelium Cycle

The stage frequencies are shown in Table 2. The last stage is always the shortest, and stages I, V, and VI are the longest. The staining frequencies for *Sorex* and *Crocidura* are given in Table 3 and Table 4.

According to the method of Rosiepen et al. [40, 41] (equation 3), we estimated the cycle duration in *S. araneus* as 8.47 ± 0.26 days (Table 1). The observed variation was from

TABLE 2. Stage frequencies of the seminiferous epithelium cycle.

Parameter	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	Stage VIII[b]	Stage IX[c]	Stage X[d]
Sorex araneus										
Percentage	17.6 ± 2.6	6.6 ± 2.1	8.9 ± 1.6	9.5 ± 2.5	16.8 ± 3.0	16.5 ± 2.6	7.5 ± 2.9	6.5 ± 1.9	7.6 ± 1.8	2.5 ± 1.0
Duration (day)[a]	1.5	0.6	0.8	0.8	1.4	1.4	0.6	0.6	0.7	0.2
Crocidura russula										
Percentage	14.7 ± 3.0	7.9 ± 2.0	6.9 ± 3.1	9.4 ± 2.5	12.9 ± 2.6	21.5 ± 4.5	9.1 ± 2.7	10.2 ± 3.3	5.3 ± 2.5	2.1 ± 1.0
Duration (day)[a]	1.8	1.0	0.9	1.2	1.6	2.7	1.1	1.3	0.6	0.3

[a] Duration calculated from the cycle length inferred from linear regression.
[b] For *Crocidura russula*, stages VIII+IX in the scheme used by Adachi [22].
[c] For *Crocidura russula*, stages X+XI in the scheme used by Adachi [22].
[d] For *Crocidura russula*, stage XII in the scheme used by Adachi [22].

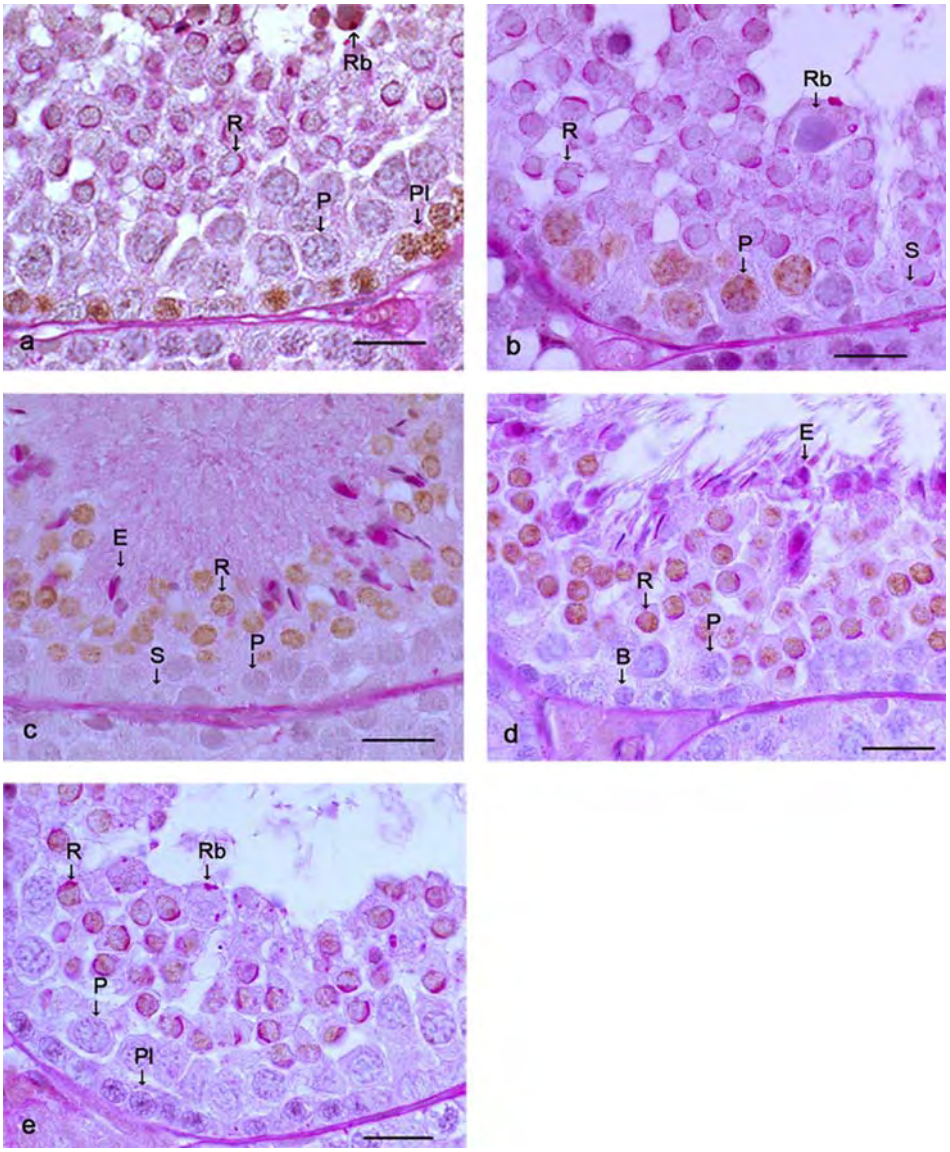


FIG. 1. The most-advanced labeled germ cells in *S. araneus* observed at different time-points after BrdU injection. Three hours after BrdU injection, preleptotene spermatocytes at stage VI (**a**). Eight days and 3 h after injection, pachytene spermatocytes at stage VI (**b**). Twelve days and 3 h after injection, spermatids at stage I (**c**). Sixteen days and 3 h after injection, spermatids at stage V (**d**) and stage VI (**e**). B, B spermatogonia; P, pachytene spematocytes; Pl, preleptotene spermatocytes; R, round spermatids; E, elongated spermatids; S, Sertoli cells; Rb, residual bodies. Bars = 20 μm.

TABLE 3. Staining frequencies of seminiferous epithelium in *Sorex araneus*.

		Stage VI		Stage V		Stage I	
Time after injection	Animal no.	Staining frequency (%)	Stage frequency (%)	Staining frequency (%)	Stage frequency (%)	Staining frequency (%)	Stage frequency (%)
3 h[a]	1	94.0	16.5				
	3	78.6	16.5				
	6	64.0	16.5				
8 day 3 h[b]	2	70.4	16.5				
	4	68.5	16.5				
	7	27.4	16.5				
12 day 3 h[c]	10					68.3	17.6
	11					73.9	17.6
	12					81.3	17.6
16 day 3 h[d]	5			71.3	16.78		
	8			84.2	16.78		
	9	45.0	16.5				

[a] Most advanced BrdU-labeled cells were preleptotene spermatocytes.
[b] Most advanced BrdU-labeled cells were pachytene spermatocytes.
[c] Most advanced BrdU-labeled cells were newly formed round spermatids.
[d] Most advanced BrdU-labeled cells were round spermatids.

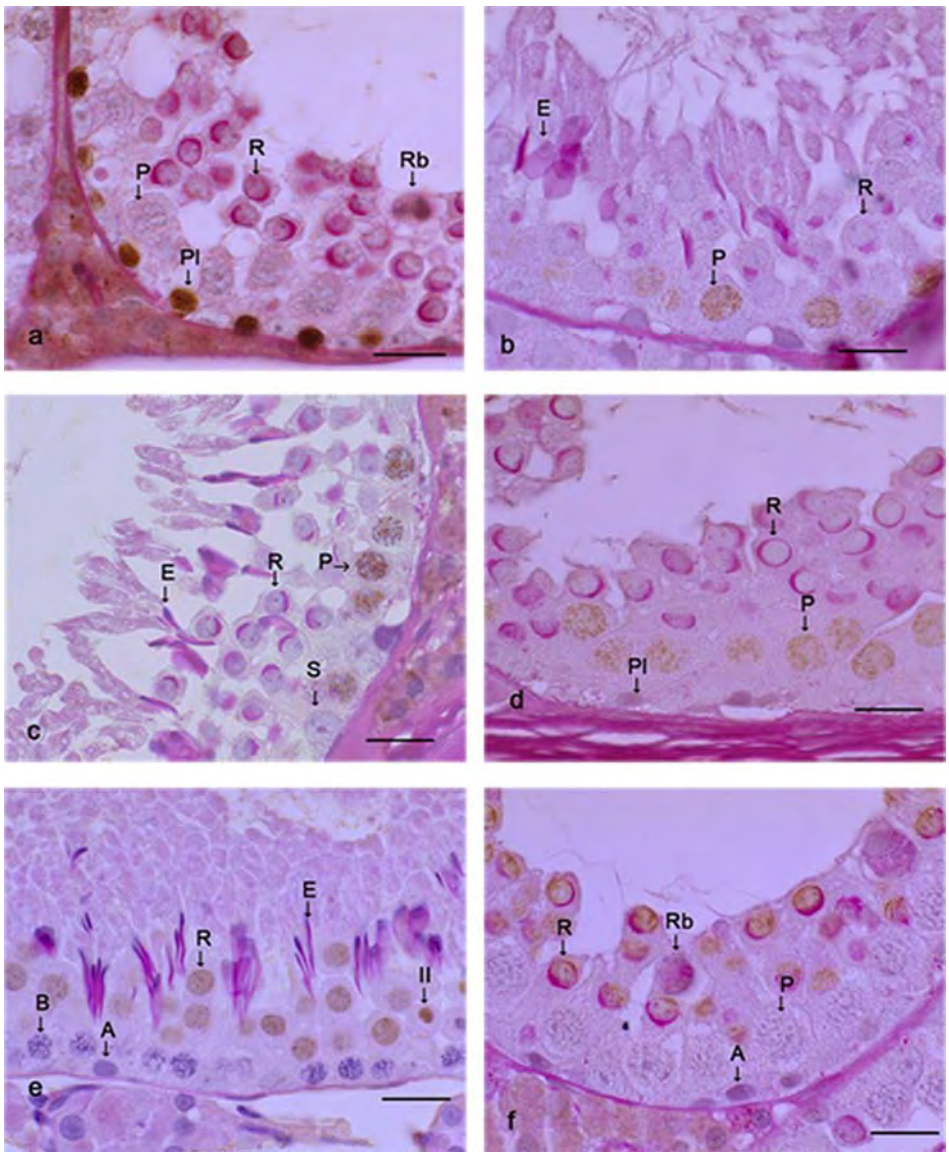


FIG. 2. The most-advanced labeled germ cells in *C. russula* observed at different time-points after BrdU injection. Three hours after BrdU injection, preleptotene spermatocytes at stage VI (**a**). Eight days and 3 h after injection, pachytene spermatocytes at stage III (**b**) and stage IV (**c**). Twelve days and 3 h after injection, pachytene spermatocytes at stage VI (**d**). Sixteen days and 3 h after injection, spermatids at stage I (**e**). Twenty-four days and 3 h after injection, spermatids at stage VI (**f**). B, B spermatogonia; P, pachytene spematocytes; Pl, preleptotene spermatocytes; R, round spermatids; E, elongated spermatids; S, Sertoli cells; Rb, residual bodies; II, secondary spermatocytes; A, A spermatogonia. Bars = 20 μm.

8.11 days to 8.79 days. In *C. russula*, the mean cycle duration was 12.42 ± 0.60 days (Table 1), varying from 11.72 days to 13.40 days.

With the second method, we estimated the duration of the seminiferous epithelium cycle by linear regression. For example, in *S. araneus* #1 at 3 h after BrdU injection, the most-advanced labeled cells were at a position that corresponded to 74.96% of the relative duration of the cycle, i.e., the sum of the stage frequencies (I+V) + (staining frequency × stage frequency of stage VI). At 8 days 3 h (195 h) after BrdU injection, the most-advanced labeled cells in another animal (#2) were at stage VI, corresponding to 171.76% of the relative duration. The label passed through almost 100% of one cycle of spermatogenesis. At 12 days 3 h (291 h), the most-advanced labeled cells in animal #10 were at 212.01% from the starting point. We also analyzed the position of the most-advanced labeled cells at 16 days post-BrdU injection. We applied the same method to the determination of the cycle duration in *C. russula*.

The cycle durations obtained by linear regression were 8.35 ± 0.13 days ($r^2 = 0.998$) for *S. araneus* and 12.12 ± 0.19 days ($r^2 = 0.997$) for *C. russula* (Fig. 3). As a consequence, the duration of spermatogenesis based on 4.5 cycles is approximately 37.6 days for *S. araneus* and 54.7 days for *C. russula*.

DISCUSSION

Technical Considerations

The present study is the first to estimate the cycle length of spermatogenesis in shrews and to apply linear regression as a new method for cycle length determination. Comparing the new linear regression method with the traditional method, the former calculation gave, as expected, a mean that was similar to that provided by the latter. However, with regard to variance, and even with a relatively small sample size (12 and 15 individuals), the new method seems to be more precise. Indeed, our results show that the regression method is significantly more accurate for both *Sorex* ($F = 0.243$, df1 11, df2 8, $P = 0.017$) and *Crocidura* ($F = 0.097$, df1 14, df2 10, $P < 0.001$). Moreover, while the classical method deals with pairs, making each value dependent upon at least two animals, the regression method allows the inference of information for each animal tested. Thus, it is possible to compare factors, such as basal metabolic rates or testosterone levels, with the residuals of the regression, thereby generating a score for spermatogenesis

TABLE 4. Staining frequencies of seminiferous epithelium in *Crocidura russula*.

Time after injection	Animal no.	Stage VI		Stage III (IV)		Stage I	
		Staining frequency (%)	Stage frequency (%)	Staining frequency (%)	Stage frequency (%)	Staining frequency (%)	Stage frequency (%)
3 h[a]	1	64.6	21.5				
	2	53.3	21.5				
	7	69.3	21.5				
	8	77.9	21.5				
8 day 3 h[b]	3			95.0	6.9		
	4			17.3[e]	9.4[e]		
	13			53.7	6.9		
12 day 3 h[b]	9	22.1	21.5				
	10	47.3	21.5				
	14	66.7	21.5				
16 day 3 h[c]	5					21.2	14.7
	6					18.1	14.7
	15					19.0	14.7
24 day 3 h[d]	11	30.0	21.5				
	12	50.9	21.5				

[a] Most advanced BrdU-labeled cells were preleptotene spermatocytes.
[b] Most advanced BrdU-labeled cells were pachytene spermatocytes.
[c] Most advanced BrdU-labeled cells were newly formed round spermatids.
[d] Most advanced BrdU-labeled cells were round spermatids.
[e] Most advanced labeled cells in animal number 4 were in stage IV.

speed with regard to the mean speed for each animal tested. This new method also allows the detection of any abnormalities in spermatogenesis speed, as individuals that diverge from the regression line are depicted graphically. For all of these reasons, we conclude that determination of the spermatogenesis cycle using this new method is statistically more precise and allows more possibilities than the traditional method.

Spermatogenesis Cycle Length Comparisons

The aim of the present study was to determine the durations of the seminiferous epithelium cycles in shrews with different metabolic rates (Soricinae versus Crocidurinae) and different mating systems (*S. araneus* versus *C. russula*). In mammals, the duration of spermatogenesis is considered to be species-specific, although strain and breed differences are found among members of the same species [24]. However, the duration of spermatogenesis is not necessarily the same for closely related species [24, 43]. Spermatogenic cycle length is under the control of the germ cell genotype [44] and is not altered by Sertoli cells or by exposure to the gonadotropic hormone environment of a different species. Recently, it has been reported that the length of the spermatogenic cycle is conserved in testis tissues xenografted from pigs and sheep into recipient mice [45]. According to our working hypothesis, the shorter cycle length of spermatogenesis in *S. araneus* than in *C. russula* should be attributable to the higher metabolic rate or the promiscuous mating system in the former. We confirmed that the cycle length of *S. araneus* (8.35 ± 0.13 days) was 68.7% of the cycle length of *C. russula* (12.12 ± 0.19 days). Therefore, with spermatogenesis of 4.5 cycles, total spermatogenesis lasts 37.6 days in *S. araneus* and 54.7 days in *C. russula*.

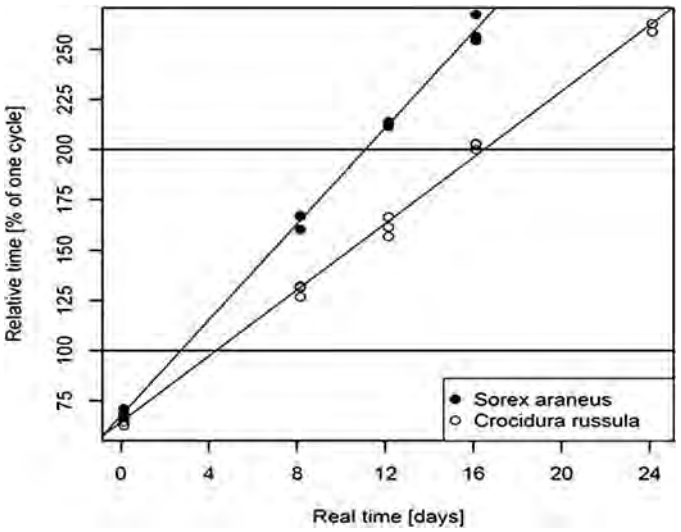


FIG. 3. Diagram showing the relationships between elapsed real time in days after injection and localization of BrdU among the stages of seminiferous epithelium expressed as relative time in percentage of the cycle. The slopes indicate the speed of spermatogenesis. Open symbols represent *S. araneus* and closed symbols represent *C. russula*.

This hypothesis of an influence of metabolic rate on cycle length is in agreement with the hypothesis of MacNab [14] with regard to the intensity of tissue synthesis and growth rate. A higher metabolic rate in mammals is correlated with a high body temperature. As in all shrews, the testes lie in a cremaster sac in the abdominal cavity [46], so body temperature may act directly on cycle length, as shown experimentally for mice [47]. A high metabolic rate is possible only in temperate or cold climates, otherwise overheating would occur. This situation is well-known in *Sorex*, which has a body temperature of about 39°C [48] and a holarctic distribution. In contrast, *Crocidura* has a lower metabolic rate [15, 17] and a lower body temperature of about 35.5°C [48], and originally had a rather tropical distribution. According to Vogel [15], the high metabolic rate probably evolved to ensure strong heating competence in a temperate climate under sufficient nutritional conditions. Tropical shrews developed a lower metabolic rate to avoid overheating [14], particularly desert shrews, such as *C. viaria* [19].

Until now, a correlation between the rate of sperm production and the metabolic rate has not been demonstrated, and our present results do not reveal the underlying mechanism. If this relationship holds true, there should also be a relationship between sperm production rate and body size, which shows a strong correlation with metabolic rate. More

data are needed to understand how metabolic rate and body mass influence the cycle length of spermatogenesis.

According to the Parker paradigm of sperm competition [25], the mating system is of primary importance in sperm production. When the frequency of copulation is higher, the testes are larger (multiple mating systems), and when copulation is rare, the testes are small (single mating systems). Apparently, the selective pressure of multiple insemination and competition among spermatozoa in the female genital tract (sperm competition) has led to the evolution of larger testes in species with multiple mating systems [49]. Larger testes have a greater volume of seminiferous tissue, which is required to achieve higher levels of sperm production. There is a strong relationship between the mating system and relative testis size, with polyandrous species tending to have relatively larger testes [26, 50].

Our results show that both increased testis size and increased speed of spermatogenesis lead to increased sperm production. As a rough estimation, sperm production in *S. araneus* could be six-fold higher than in *C. russula*, due to the volume difference (a factor of 4.25) multiplied by the speed difference (a factor of 1.45). A similar finding in Australian mice has been reported by Peirce and Breed [51]. In that study, the promiscuous *Pseudomys australis* had a relative testis size of 3.21 and a cycle length of 11.2 days, whereas *Notomys alexis* had a relative testis size of only 0.16 and a cycle length of 14 days. This was interpreted by the authors as the result of a single-male mating system [51].

Finally, this study raises questions as to the plesiomorphic situation and the derived situation. Evolution can go in either direction, depending on the selective forces. Sperm production is certainly energy-consuming, and a lack of sperm competition may quickly lead to a reduction in sperm production. On the other hand, species that replace sperm competition with mate-guarding or even paternal investment in the survival of the litter, as is the case for *C. russula* [33], may simply invest their energy differently, while a trade-off may lead to equal fitness.

To our knowledge, basal metabolic rate and spermatogenic cycle length have not been shown to be correlated, and our present investigation was not designed to reveal such a relationship. Indeed, the speed of the cycle may depend on different physiological mechanisms. To separate the effect of metabolic rate from the effect of the mating system, future studies of sperm cycle length should include, on the one hand, several Soricidae that have low metabolic rates (e.g., *Notiosorex* [52]) or that lack multiple paternity, and on the other hand, some *Crocidura* with multiple paternity, if such types exist in nature.

ACKNOWLEDGMENTS

We thank R. V. Krstic, S. Kasas and C. Wedekind for critical reading of the manuscript and Anne-Marie Mehmeti for the provision of laboratory facilities.

REFERENCES

1. Leblond CP, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann N Y Acad Sci 1952; 55:548–573.
2. Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neil JD (eds.), The Physiology of Reproduction. New York: Raven Press; 1994:1363-1434.
3. França LR, Becker-Silva SC, Chiarini-Garcia H. The length of the cycle of seminiferous epithelium in goats (*Capra hircus*). Tissue Cell 1999; 31: 274–280.
4. Leal MC, França LR. The seminiferous epithelium cycle length in the black tufted-ear marmoset (*Callithrix penicillata*) is similar to humans. Biol Reprod 2006; 74:616–624.
5. Crocock CA, Clark JR. Duration of spermatogenesis in the vole (*Microtus agrestis*) and bank vole (*Clethrionomys glareolus*). J Reprod Fertil 1976; 47:133–135.
6. Oud JL, De Rooij DG. Spermatogenesis in the Chinese hamster. Anat Rec 1977; 187:113–124.
7. Dang DC. Durée du cycle de l'épithélium séminifère du singe crabier, *Macaca fascicularis*. Ann Biol Anim Biochem Biophys 1971; 11:373–377.
8. Heller CG, Clermont Y. Spermatogenesis in man: an estimate of its duration. Science 1963; 140:184–185.
9. Setchell BP, Carrick FN. Spermatogenesis in some Australian marsupials. Aust J Zool 1973; 21:491–499.
10. Queiroz GF, Nogueira JC. Duration of the cycle of the seminiferous epithelium and quantitative histology of the testis of the South American white-belly opossum (*Didelphis albiventris*), Marsupialia. Reprod Fertil Dev 1992; 4:213–222.
11. Schmidt-Nielsen K. Scaling. Why Is Animal Size So Important? Cambridge: Cambridge University Press; 1984.
12. Calder WA. Size, Function, and Life History. Cambridge: Harvard University Press; 1984.
13. Peters RH. The Ecological Implications of Body Size. Cambridge; Cambridge University Press; 1983.
14. McNab BK. Food habits, energetics, and the population biology of mammals. The American Naturalist 1980; 116:106–124.
15. Vogel P. Energy consumption of European and African shrews. Acta Theriol 1976; 21:195–206.
16. Nagel A. Sauerstoffverbrauch, Temperaturregulation und Herzfrequenz bei europäischen Spitzmäuse (Soricidae). Z Säugetierkunde 1985; 50:249–266.
17. Genoud M. Energetics strategies of shrews: ecological constraints and evolutionary implications. Mammal Rev 1988; 18:173–193.
18. Nagel A. Sauerstoffverbrauch, Temperaturregulation und Herzfrequenz der europäischen Spitzmäuse (Soricidae, Mammalia). Tübingen, Germany: Eberhard-Karls-Univerisität Tübingen; 1980. Thesis.
19. Sparti A. Comparative temperature regulation of African and European shrews. Comp Biochem Phys 1990; 97:391–397.
20. Plöen L, Ekwall H, Afzelius BA. Spermiogenesis and the spermatozoa of the European common shrew (*Sorex araneus* L.). J Ultrastruct Res 1979; 68:149–159.
21. Garagna S, Zuccotti M, Searle J, Redi CA. Histological description of the seminiferous epithelium cycle in the common shrew (*Sorex araneus* L.). Boll Zool 1989; 56:299–303.
22. Adachi Y, Kurohmaru M, Hattori S, Hayashi Y. Spermatogenesis in the Watase's shrew, *Crocidura watasei*—a light electron microscopic study. Exp Anim 1992; 41:295–303.
23. Kurohmaru M, Kobayashi H, Hattori S, Nishida T, Hayashi Y. Spermatogenesis and ultrastructure of a peculiar acrosomal formation in the musk shrew, *Suncus murinus*. J Anat 1994; 185:503–509.
24. Russell LD, Ettlin RA, Sinha-Hikim AP, Clegg ED. Mammalian spermatogenesis, In: Russell LD, Ettlin RA, Sinha-Hikim AP, Clegg ED (eds.), Histological and Histopathological Evaluation of the Testes. Clearwater, FL: Cache River Press; 1990:1–40.
25. Parker GA. Sperm competition and its evolutionary consequences in the insects. Biol Rev 1970; 45:525–567.
26. Kenagy GJ, Trombulak SC. Size and function of mammalian testes in relation to body size. J Mammal 1986; 67:1–22.
27. Møller AP. Ejaculate quality, testes size and sperm competition in primates. J Human Evol 1988; 17:479–488.
28. Møller AP. Ejaculate quality, testes size and sperm production in mammals. Func Ecol 1989; 3:91–96.
29. Freeman S. The evolution of the scrotum: A new hypothesis. J Theor Biol 1990; 145:429–445.
30. Searle JB. Evidence for multiple paternity in the common shrew (*Sorex araneus*). J Mammal 1990; 71:139–144.
31. Tegelstrom H, Searle JB, Brookfield J, Mercer S. Multiple paternity in wild common shrew (*Sorex araneus*) is confirmed by DNA fingerprinting. Heredity 1991; 66:373–379.
32. Stockley P, Searle JB, Macdonald DW, Jones CS. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. Proc Roy Soc Lond 1993; B254:173–179.
33. Cantoni D, Vogel P. Social organisation and mating system of free-ranging, greater white-tooted shrews, *Crocidura russula*. Anim Behav 1989; 38:205–214.
34. Bouteiller C, Perrin N. Individual reproductive success and effective population size in the greater white-toothed shrew *Crocidura russula*. Proc Roy Soc B-Biol Sci 2000; B267:701–705.
35. Besançon F. Contribution à l'étude de la biologie et de la stratégie de

reproduction de *C. russula* (Soricidae, Insectivora) en zone tempérée. Lausanne, Switzerland: University of Lausanne; 1984. Thesis.
36. Vogel P. Metabolic levels and biological strategies in shrews. In: Schmidt-Nielsen K, Bolis L, Taylor CR (eds.), Comparative Physiology: Primitive Mammals. Cambridge: Cambridge University Press; 1980:170–180.
37. Genoud M, Vogel P. Energy requirements during reproduction and reproductive effort in shrews (Soricidae). J Zool 1990; 220:41–60.
38. Brünner H, Hausser J. Genetic and karyotypic structure of a hybrid zone between the chromosomal races Cordon and Valais in the common shrew, *Sorex araneus*. Hereditas 1996; 125:147–158.
39. Cosson JF, Hutterer R, Libois R, Sara M, Taberlet P, Vogel P. Phylogeographical footprints of the Strait of Gibraltar and Quaternary climatic fluctuations in the western Mediterranean: a case study with the greater white-toothed shrew, *Crocidura russula* (Mammalia: Soricidae). Mol Ecol 2005; 14:1151–1162.
40. Rosiepen G, Weinbauer GF, Schlatt S, Behre HM, Nieschlag E. Duration of the cycle of the seminiferous epithelium, estimated by the 5-bromodeoxyuridine technique, in laboratory and feral rats. J Reprod Fertil 1994; 100:299–306.
41. Rosiepen G, Arslan M, Clemen G, Nieschlag E, Weinbauer GF. Estimation of the duration of the cycle of the seminiferous epithelium in the non-human primate *Macaca mulatta* using the 5-bromodeoxyuridine technique. Cell Tissue Res 1997; 288:365–369.
42. Duarte LC, Bouteiller C, Fontanillas P, Petit E, Perrin N. Inbreeding in the greater white-toothed shrew, Crocidura russula. Evol 2003; 57:638–645.
43. França LR, Russell LD. The testes of domestic mammals. In: Regadera J, Martinez-Garcia F (eds.), Male Reproduction. A Multidisciplinary Overview. Madrid: Churchill Livingstone; 1998:197–219.
44. França LR, Ogawa T, Avarbock MR, Brinster RL, Russell LD. Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol Reprod 1998; 59:1371–1377.
45. Zeng W, Avelar GF, Rathi R, Franca LR, Dobrinski I. The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J Androl 2006; 27:527–533.
46. Bedford JM, Berrios M, Dryden GL. Biology of the scrotum. IV. Testis location and temperature sensitivity. J Exp Zool 1982; 224:379–388.
47. Meistrich ML, Eng VWS, Loir M. Temperature effects on the kinetics of spermatogenesis in the mouse. Cell Tissue Kinet 1973; 6:379–393.
48. Frey H. La température corporelle de *Suncus etruscus* (Soricidae, Insectivora) au cours de l'activité, du repos normothermique et de la torpeur. R Suisse Zool 1979; 86:653–662.
49. Harvey PH, Harcourt AH. Sperm competition, testes size and breeding systems in primates. In: Smith RL (eds.), Sperm Competition And The Evolution of Animal Mating Systems. New York: Academic Press; 1984: 589–600.
50. Møller AP, Birkhead TR. Copulation behaviour in mammals: evidence that sperm competition is widespread. Biol J Linn Soc 1989; 38:119–131.
51. Peirce EJ, Breed WG. A comparative study of sperm production in two species of Australian arid zone rodents (*Pseudomys australi*s, *Notomys alexis*) with marked differences in testis size. Reproduction 2001; 121: 239–247.
52. Lindstedt SL. Energetics and water economy of the smallest desert mammal. Phys Zool 1980; 53:173–176.

ELSEVIER

Animal Reproduction Science 107 (2008) 148–160

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Histological description of seminiferous epithelium and cycle length of spermatogenesis in the water shrew *Neomys fodiens* (Mammalia: Soricidae)

Roumen Parapanov*, Sébastien Nusslé, Jacques Hausser, Peter Vogel

Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland

Received 22 February 2007; received in revised form 22 May 2007; accepted 1 June 2007
Available online 15 June 2007

Abstract

Recently, we examined the spermatogenesis cycle length in two shrews species, *Sorex araneus* characterized by a very high metabolic rate and a polyandric mating system (sperm competition) resulting in a short cycle and *Crocidura russula* characterized by a much lower metabolic rate and a monogamous mating system showing a longer cycle. In this study, we investigated the spermatogenesis cycle in *Neomys fodiens* showing an intermediate metabolic rate. We described the stages of seminiferous epithelium according to the spermatid morphology method and we calculated the cycle length of spermatogenesis using incorporation of 5-bromodeoxyuridine into DNA of the germ cells. Twelve males were injected intraperitoneally with 5-bromodeoxyuridine, and the testes were collected. For cycle length determination, we applied a recently developed statistical method. The calculated cycle length is 8.69 days and the total duration of spermatogenesis based on 4.5 cycles is approximately 39.1 days, intermediate between the duration of spermatogenesis of *S. araneus* (37.6 days) and *C. russula* (54.5 days) and therefore congruent with both the metabolic rate hypothesis and the sperm competition hypothesis. Relative testes size of 1.4% of body mass indicates a promiscuous mating system.

Keywords: BrdU; Sperm competition; Cycle of spermatogenesis; Testis size; *Neomys fodiens*

1. Introduction

Spermatogenesis is a highly organized process characterized by mitotic spermatogonial cell proliferation within the seminiferous epithelium followed by two meiotic divisions and ending by

* Corresponding author. Tel.: +41 21 692 41 54.
E-mail address: Roumen.Parapanov@unil.ch (R. Parapanov).

0378-4320/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2007.06.012

spermiogenesis. In this process, the diploid spermatocytes develop into haploid free spermatozoa (Leblond and Clermont, 1952; Russell et al., 1990). During spermatogenesis, the male germ cells are closely arranged in cellular association named stages. The complete series of changes occurring in a given area of the seminiferous epithelium between two successive appearances of the same cellular association is defined as the cycle of seminiferous epithelium (Leblond and Clermont, 1952). The time interval between two appearances of the same cell association in one area is called the duration of the cycle (Leblond and Clermont, 1952). The types of germ cell involved in this process are similar in all mammals (Sharpe, 1994; França et al., 1999). In contrast, differences occur in the topographical arrangement of the spermatogenetic stages and in the duration of spermatogenesis (Leal and França, 2006).

The duration of the cycle has been studied in many species, and seems to be rather constant within species, but varying between species. In Eutherians, the cycle length may be as short as 6.7 days in the bank vole *Clethrionomys glareolus* (Crocock and Clark, 1976) and up to 17 days in the Chinese hamster *Cricetulus griseus* (Oud and De Rooij, 1977). It is known that the spermatogenetic cycle length is controlled by the germ cell genotype (França et al., 1998) and is not influenced by Sertoli cells or by hormonal environment. In addition, it was shown that the duration of the cycle of spermatogenesis is not altered in testis tissue xenografts (Zeng et al., 2006). Within a species, certain drug applications may slightly modify the cycle length (Weinbauer et al., 1998), and an increase of the testis temperature is able to shorten the cycle length, as experimentally shown for mice (Meistrich et al., 1973). However, it is not clear which are the proximate factors (physiological mechanisms of regulation) and ultimate factors (selective pressures affecting the evolution of the trait) responsible for variation of cycle length between species.

Recently, two new factors which might influence the cycle length were reported: sperm competition and metabolic rate intensity. Peirce and Breed (2001) showed in Australian rodents that *Notomys alexis*, a species with a single male mating system, has a cycle length of 14 days whereas *Pseudomys australis*, a species with a promiscuous mating system, has a cycle length of 11.2 days. The shorter cycle leads to more rapid sperm production and could reflect an adaptation to competition between males. On the other hand, the metabolic rate may also be implicated in the regulation of the spermatogenetic cycle length as hypothesized by Parapanov et al. (2007) in their comparison of different shrew species. Indeed, the greater white-toothed shrew *Crocidura russula* showing a low metabolic rate (129% of the size expected value) has a cycle length of 12.1 days whereas the common shrew *Sorex araneus* showing a high metabolic rate (250–350% of the size expected value) has a cycle length of only 8.4 days. A final interpretation is yet not possible because the metabolic rate is related to differences in body temperature (39 °C in *Sorex*, 35.5 °C in *Crocidura*) that might be one of the proximate factors. Moreover, we have in *C. russula* a monogamous mating system (Cantoni and Vogel, 1989) without sperm competition, while in *S. araneus* we have, within each litter, multiple paternity resulting in sperm competition (Searle, 1990). Therefore, at least theoretically, a higher metabolic rate could be a proximate factor to increase sperm production as an evolutionary adaptation with regard to a particular mating system. Obviously, our knowledge is still too limited and further species have to be investigated in order to evaluate these hypotheses.

Here, we investigated for the first time the spermatogenesis cycle in the water shrew, *Neomys fodiens* (Pennant, 1771), describing the stages of seminiferous epithelium according to the spermatid morphology method. Spermatogenetic cycle length was examined using incorporation of 5-bromodeoxyuridine into DNA of the germ cells. An additional aim was to use the data to explore further relationships between spermatogenetic cycle characteristics, metabolic rate and mating system.

2. Materials and methods

2.1. Animals

N. fodiens is an insectivorous mammal widespread in Europe. Twelve adult males weighting 16.8 ± 1.5 g were captured in the period from April to June 2005–2006 in the region of Champtauroz FR, Switzerland (altitude 800 m). The animals were kept in a building offering a roof protecting them from direct rain, but with walls of wire mesh that exposed the shrews to natural fluctuation of temperature and humidity according to the weather. In such a stimulating environment, wild shrews show much better body conditions than in an indoor laboratory confinement (Vogel, 1990). The shrews were caged individually on natural soil. They had free access to water and to food composed of minced horsemeat with vitamin supplement, pieces of fish, and some mealworms.

2.2. Administration of BrdU

5-Bromodeoxyuridine (BD Deutschland) was administered as a single *i.p.* injection of a dose of 50 mg/kg for each animal. Following this injection, groups of three animals were killed by halothane overdose at 3 h, at 8 days 3 h, at 12 days 3 h and at 16 days 3 h. The testes were removed, weighted and fixed in 4% solution of paraformaldehyde for 3 days, dehydrated in a graded series of ethanol and embedded in Paraplast. The histological sections (3 μm) of the testes were dewaxed and rehydrated. BrdU was localized using the ZYMED® BrdU Staining Kit provided by Invitrogene, USA. Endogenous peroxidase was inactivated in 3% (v/v) H_2O_2 in methanol for 10 min at room temperature. To expose 5-bromodeoxyuridine for immunohistochemical localisation, DNA was denatured in 0.17% (v/v) trypsin solution for 10 min at 37 °C in a moist chamber. Incubation with a biotinylated anti-BrdU antibody was performed for 1 h at room temperature. Sections were then incubated with Streptavidin-HRP for 10 min at room temperature. DAB substrate solution was added to cover the tissue sections and incubated for 5 min or until the colour intensity is developed. PAS-haematoxylin-staining was performed for identification of spermatogenetic stages. Incubation with 1% periodic acid (for 10 min at room temperature) and staining with Schiff's reagent (for 30 min at room temperature) was following by incubation with Mayer's haematoxylin solution (5 min at room temperature). Histological preparations were observed under microscope Axiophot ZEISS, Germany.

2.3. Stages and stage frequencies of the cycle of seminiferous epithelium

The 10 stages of the cycle of seminiferous epithelium were characterized according to the morphological characteristics of spermatids and its acrosomic system development.

The relative stage frequencies of each stage of the cycle were determined from the testes sections counterstained with PAS–haematoxylin. We counted 200–400 round tubular cross-sections per animal. A total number of 5400 tubules from 12 *N. fodiens* were scored.

Stage frequencies were calculated in each animal using the following equation:

$$\text{Stage frequency}(\%) = \frac{\text{number of tubules of given stage}}{\text{number of all tubules}} \times 100 \quad (1)$$

A mean value was calculated for the frequency of each stage.

2.4. BrdU staining frequency

The estimation of staining frequency was based on the percentage of tubules of a certain stage, containing the most advanced germ cells labelled by the BrdU as follows:

$$\text{Staining frequency}(\%) = \frac{\text{number of tubules containing the most advanced labeled cells in a given stage}}{\text{number of all tubules of that stage}} \times 100 \quad (2)$$

We determined the staining frequency scoring more than 50 tubules cross-section for each animal.

2.5. Duration of the cycle of seminiferous epithelium

We estimated the duration of the cycle of seminiferous epithelium by linear regression according to Parapanov et al. (2007) which gave, in a comparative test, the most precise result. The regression function used was $Y = aX + b$. Y is the proportion (%) of cycle length reached at a given time point from BrdU injection. X is the length of real time (days) elapsed since the injection. b is the intercept which is the percentage of the cycle that would have been stained at real time $T = 0$.

The duration of one cycle (100% relative time) was inferred from the regression function and is given as the result used for comparisons. The standard deviation was also inferred from the linear regression.

2.6. Statistical analysis

All data are presented as mean ± SE. Analysis of regression was performed using software R (R Development Core Team, 2005). The significance level was considered to be $\alpha = 0.05$.

2.7. Ethical considerations

This project was performed under authorisation of State commission for animal experiments of Canton de Vaud. The number of samples was kept as low as possible because all species of shrews are protected animals in Switzerland.

3. Results

3.1. Body mass, testicular weight and relative testis size

Administration of the BrdU was well tolerated and negative effects were not observed during the period after injection. The body mass was 16.8 ± 1.5 g, the absolute testes mass was 0.241 ± 0.041 g and relative testis size was 1.4% ± 0.197 (the mass of the two testes as percentage of body mass).

3.2. Stages of seminiferous epithelium cycle

To date, the stages of spermatogenesis in the water shrew have never been described. We present a detailed and illustrated description of them (Fig. 1 and Fig. 2).

Stage I starts at the end of the second meiotic division of the secondary spermatocytes from which newly formed round spermatids arise. These spermatids are characterized without or with

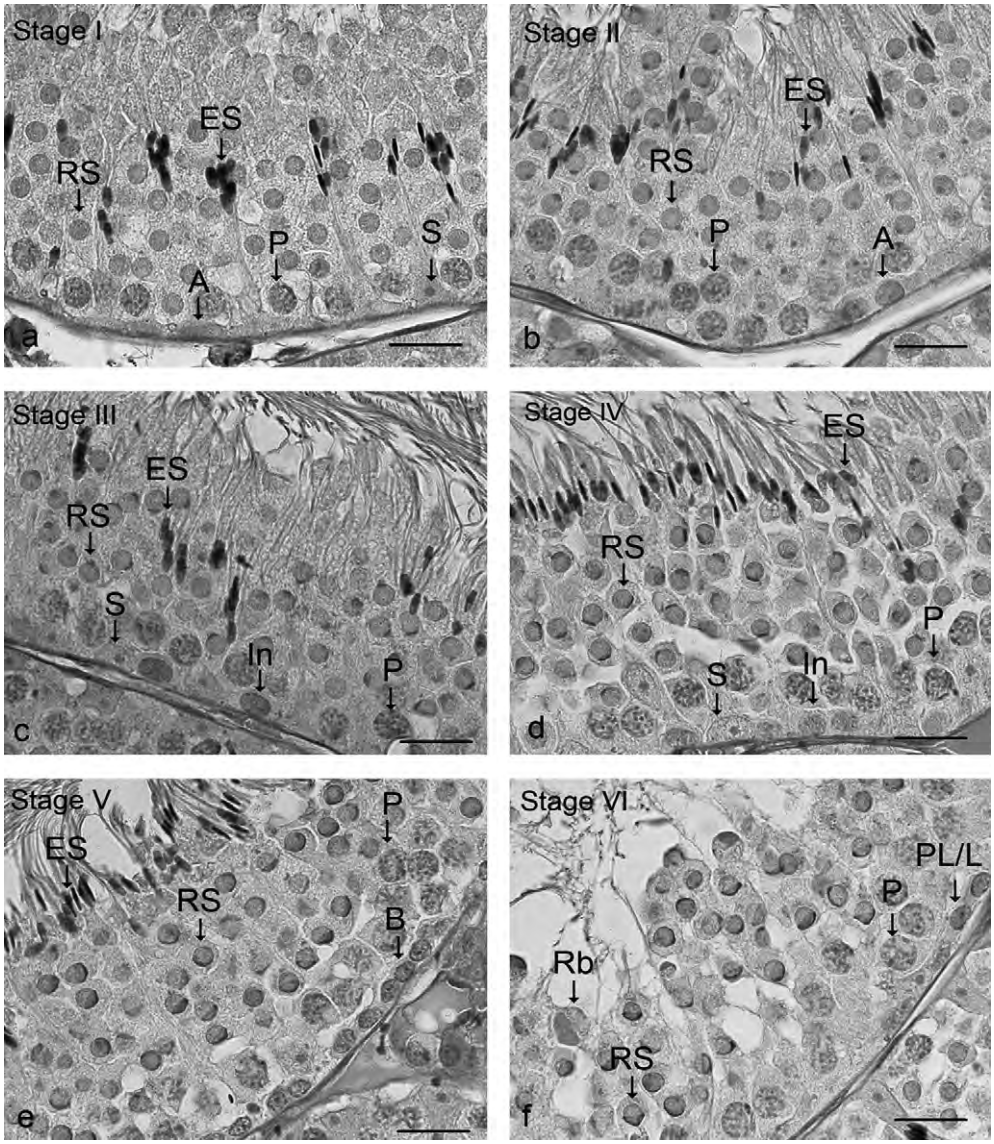


Fig. 1. (a–f) Stage I to VI of spermatogenesis cycle of *N. fodiens*. Abbreviations: A spermatogonia (A), intermediate spermatogonia (In), B spermatogonia (B), pachytene spermatocytes (P), preleptotene–leptotene spermatocytes (PL/L), round spermatids (RS), elongated spermatids (ES), Sertoli cells (S), residual bodies (Rb). Bars = 20 μm.

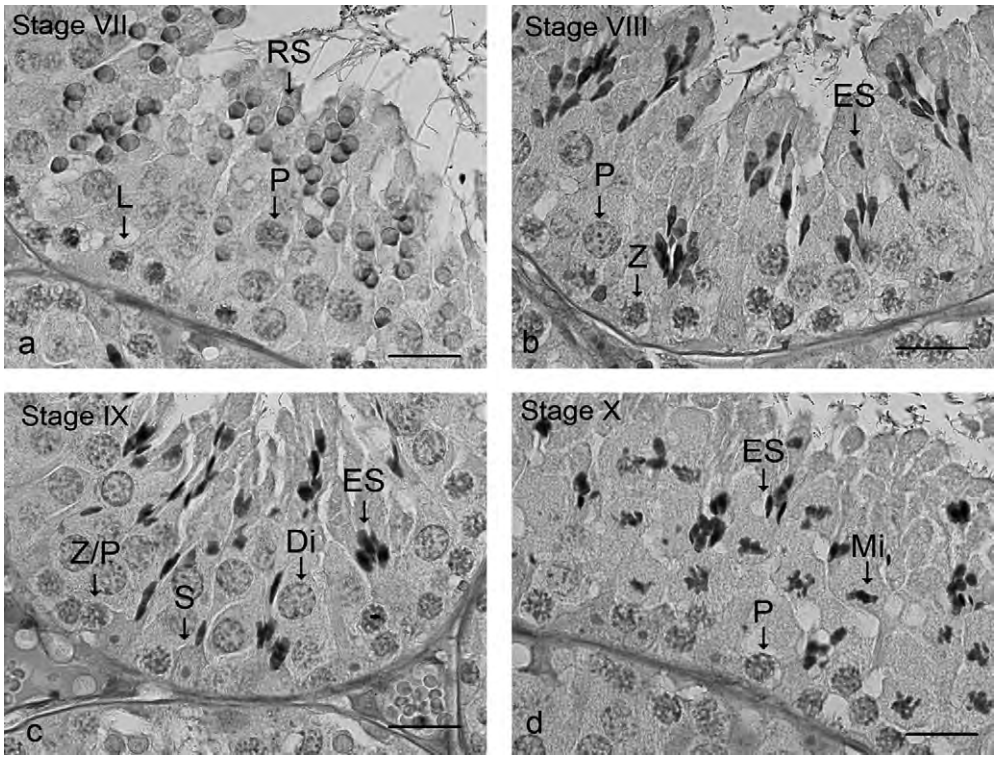


Fig. 2. (a–d) Stage VII to X of spermatogenesis cycle of *N. fodiens*. Abbreviations: Leptotene spermatocytes (L), zigotene spermatocytes (Z), zigotene–pachytene spermatocytes (Z/P), pachytene spermatocytes (P), diplotene spermatocytes (Di), meiotic figures (Mi), round spermatids (RS), elongated spermatids (ES), Sertoli cells (S), residual bodies (Rb). Bars = 20 μm.

a few proacrosomic granules in the vicinity of the nucleus (step 1). The elongated spermatids in maturation phase were located in the luminal surface slightly embedded into the seminiferous epithelium (step 11). However, their cytoplasmic bridges still relate them with the Sertoli cells. At this stage, we also observe spermatogonia type "A" with fine dust like chromatin in the oval nucleus. Above these, there are spermatocytes in early pachytene stage (Fig. 1a).

Stage II is characterized by the fusion of the proacrosomic vesicles and the development of a single large acrosomic granule over the nucleus in the round spermatids (step 2). The elongated spermatids are located as in stage I. The pachytene spermatocytes show progressive growth (Fig. 1b).

The acrosomal granule starts to flatten on the surface of the spermatid nucleus (step 3). In the elongated spermatids the process of the flattening of nucleus continues and reaches its maximum (step 12). Pachytene spermatocytes continue to increase in size. In addition to the type "A" spermatogonia, we detected a new type of spermatogonia, intermediate, appearing in the basement compartment of tubule (Fig. 1c).

Stage IV is marked by laterally extension of the acrosomic granule over the nucleus surface of the round spermatids (step 4). In the luminal surface of the seminiferous epithelium, there are maturing spermatids with few cytoplasm (step 12). Type "A" and "In" spermatogonia are present in the basement compartment. The pachytene spermatocytes continue to increase in size (Fig. 1d).

At stage V, the acrosome covers half of the nuclear surface of round spermatids (step 5).

The mature spermatids without the cytoplasm (step 13) are arranged along the luminal surface of the epithelium ready to be released. In the basal compartment, type B spermatogonia are visible. The pachytene spermatocytes in the middle region of the epithelium are present (Fig. 1e).

After the release of mature spermatids into the lumen, only one generation of spermatids (step 6) is present in the epithelium. The residual bodies remain within the seminiferous epithelium. The acrosome surrounds more than half of the nuclear surface. In the middle region, there are pachytene spermatocytes. The spermatocytes at the transition phase preleptotene–leptotene with a small nucleus are present in the basal compartment (Fig. 1f).

At stage VII, the round spermatids (step 7) begin to change their orientation and rotate their acrosome toward to the basement membrane and their cytoplasm appears elongated in opposite parts of the nucleus. The residual bodies disappear. In the basal compartment, leptotene spermatocytes are detectable, together with, in middle compartment pachytene spermatocytes (Fig. 2a).

The elongated spermatids (step 8) start to form the bundles and somewhat penetrating into direction of the Sertoli cells. Two generation of spermatocytes are present. Pachytene spermatocytes show a larger nucleus than the spermatocytes at zygotene phase arranged in the vicinity of the basement membrane (Fig. 2b).

The acrosome of spermatids (step 9) is extending towards the base of the seminiferous epithelium. The spermatid nucleus is elongated and flattening. The nucleus sizes of spermatocytes continue to increase and they are at the transition phase between pachytene and diplotene phases. Spermatocytes at transition phase zygotene–pachytene are detected in vicinity of the basement compartment (Fig. 2c).

At this sage, the spermatid nucleus is partially covered by the acrosome and they have almost the final shape (step 10). In addition, this stage characterized by the presence of meiotic figures results of divisions of primary or secondary spermatocytes. The younger generation of primary spermatocytes was differentiated into the early pachytene phase of the meiotic prophase (Fig. 2d).

3.3. BrdU immunohistochemistry

At 3 h after BrdU injection, the label was localized in the nuclei of preleptotene/leptotene spermatocytes in stage VI (Fig. 3a). At 8 days 3 h, the most advanced cells containing BrdU were pachytene spermatocytes in the same stage (Fig. 3b). At 12 days 3 h, the most advanced BrdU-labelled cells in one animal were newly formed round spermatids in stage I (Fig. 3d) and in the dividing cells in stage X in two animals (Fig. 3c). At 16 days 3 h, the most advanced BrdU-labelled cells were round spermatids in stage V (Fig. 2f).

3.4. Stage frequencies, staining frequency and cycle length of seminiferous epithelium

The mean percentage of stage frequencies is shown in Table 1. The estimation of staining frequency was based on the percentage of tubules of a given stage, containing the most advanced germ cells labelled by the BrdU divided by all tubules in the same stage. Staining frequencies are indicated in Table 2.

The cycle length obtained by the linear regression was 8.69 ± 0.14days ($r^2 = 0.997$) (Fig. 4). Thus, in *N. fodiens* the total duration of spermatogenesis based on 4.5 cycles is approximately 39.1 days

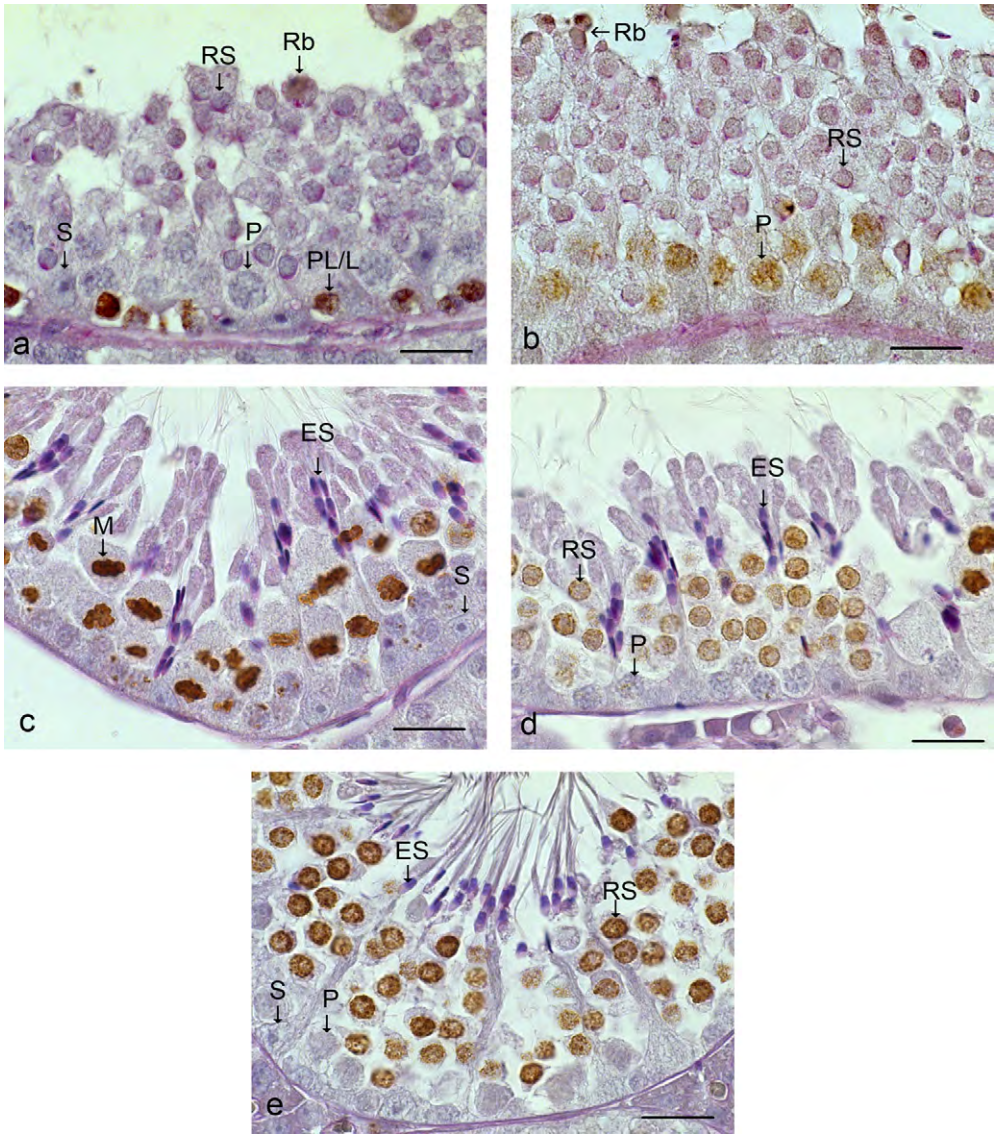


Fig. 3. Most advanced labelled germ cells in *N. fodiens* observed at different time periods after BrdU-injection. Three hours after BrdU injection, preleptotene spermatocytes at stage VI (a). Eight days and 3 h after injection, pachytene spermatocytes at stage VI (b). Twelve days and 3 h after injection, spermatids at stage X (c) and I (d). Sixteen days and 3 h after injection, spermatids at stage V (e). B spermatogonia (B), pachytene spermatocytes (P), preleptotene spermatocytes (PL/L), round spermatids (RS), elongated spermatids (ES), Sertoli cells (S), residual bodies (Rb). Bars = 20 μm.

4. Discussion

The aim of this work was to describe the stages of seminiferous epithelium and to determine the cycle length of spermatogenesis in *N. fodiens* in order to increase our basic knowledge of spermatogenesis in shrews. In addition, these data contribute to the different hypothesis explaining

Table 1
Stage frequencies of the seminiferous epithelium cycle in *Neomys fodiens*

	I	II	III	IV	V	VI	VII	VIII	IX	X
%	15.0 ± 3.0	6.0 ± 1.4	7.5 ± 2.0	14.3 ± 3.2	13.3 ± 2.7	11.5 ± 2.5	7.7 ± 1.9	6.6 ± 1.8	15.1 ± 2.6	2.9 ± 0.8
Days	1.3	0.5	0.7	1.3	1.2	1.0	0.7	0.6	1.3	0.3

The duration (in days) is given as information and was calculated from the cycle length inferred from linear regression.

the differences in the cycle length of spermatogenesis in shrews. Our criteria for stage identification were based on the morphological characteristics of the spermatids, in particular their nucleus and acrosomic system (Russell et al., 1990). This method divides the cycle of spermatogenesis in more than eight stages. In water shrew, the seminiferous tubules show segmental arrangement, one stage found per tubule cross-section. This is also the situation in all shrews studied at present, i.e., the common shrew *S. araneus* (Garagna et al., 1989), the Japanese *Crocidura watasei* (Adachi et al., 1992), the Asian *Suncus murinus* (Kurohmaru et al., 1994), and greater white-tooted shrew *C. russula* (Parapanov et al., 2007). The germ cell association described in the spermatogenetic cycle in the water shrew *N. fodiens* (10 stages) are very similar to those observed previously in common shrew *S. araneus* (Garagna et al., 1989). In regard to sperm morphology, we also found similar characteristics with *S. araneus*, namely in the shape of nucleus and acrosome which is less prominent in Soricinae than in Crocidurinae (Plöen et al., 1979; Bedford et al., 1994).

Based on the hypothesis of McNab (1980) who suggested that high metabolic rate may increase cell cycle speed and tissue synthesis, we conclude that metabolic rate may be a potential factor explaining the duration of spermatogenesis (Parapanov et al., 2007). The results presented here are in agreement with this hypothesis. In regard to metabolic rate, body temperature, and cycle length of spermatogenesis, the values of *N. fodiens* are situated at an intermediate position between *S. araneus* and *C. russula*. The parameters are showed in Table 3.

According to the sperm competition theory (Parker, 1970), the mating system is of main importance for sperm production. Apparently, multiple copulation and sperm competition in the female genital tract led to the evolution of greater relative testis size in species with multiple mating systems (Harvey and Harcourt, 1984), with polyandrous species tending to have relatively larger testes (Kenagy and Trombulak, 1986; Møller and Birkhead, 1989). In this context, our results (Table 3) showed great relative testes size in *N. fodiens* (1.4%). In comparison, the relative testis size in promiscuous *S. araneus* is 1.6%, but only 0.3% in monogamous *C. russula* (Parapanov et al., 2007). The relative large testis size in *N. fodiens* suggests a multiple mating systems. This result is in agreement with the supposition made by Cantoni (1993) concerning the social behaviour of the water shrew. However, genetic tests of multiple paternities are necessary to confirm our conclusions.

In conclusion, this study shows that in *N. fodiens,* characterized by rather high metabolic rate and greater relative testes size, the cycle length of spermatogenesis of 8.69 days is relatively short. This is in agreement with our previous findings where we have shown that in shrews, the higher metabolic rate and greater relative testis size are associated with a short cycle length of spermatogenesis. However, the results do not yet allow estimating the contribution of each of the potential factors. According to McNab (1980), a higher metabolic rate evolved rather in the context of female reproductive output, litter size and developmental speed of the embryo. Therefore, the shorter cycle length in shrews, eventually mediated by higher body temperature, may only present a side effect without adaptive significance for the males.

Table 2
Staining frequencies of seminiferous epithelium in *Neomys fodiens*

Time after injection	Number of animal	Stage VI		Stage V		Stage X—I	
		Staining frequency (%)	Stage frequency (%)	Staining frequency (%)	Stage frequency (%)	Staining frequency (%)	Stage frequency (%)
Three hours[a]	1	92.4	11.5				
	2	70.4	11.5				
	72	46.4	11.5				
Eight days 3 h[b]	6	82.1	11.5				
	7	79.7	11.5				
	68	42.3	11.5				
Twelve days 3 h[c]	61*					29.0*	2.9*
	65					81.9	15.0
	69					96.4	15.0
Sixteen days 3 h[d]	8			69.1	13.3		
	10			66.1	13.3		
	70			87.8	13.3		

* In number 61, the most advanced BrdU-labelled cells were newly formed round spermatids (stage I).
[a] Most advanced BrdU-labelled cells were preleptotene/leptotene spermatocytes.
[b] Most advanced BrdU-labelled cells were pachytene spermatocytes.
[c] Most advanced BrdU-labelled cells were spermatocytes in meiotic division (stage X).
[d] Most advanced BrdU-labelled cells were round spermatids (stage V).

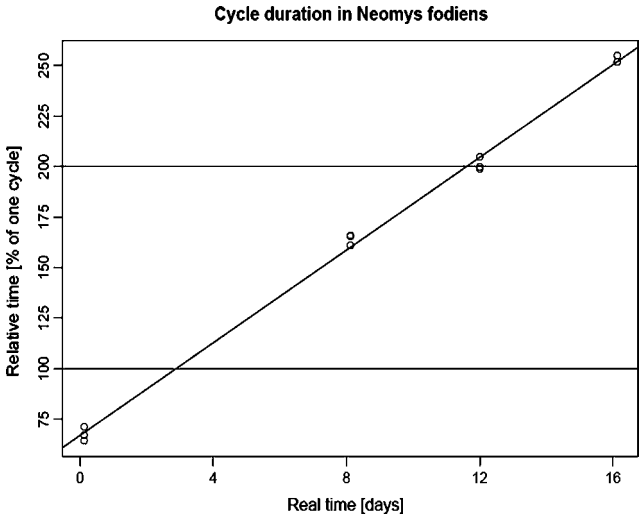


Fig. 4. Diagram showing the relationships between elapsed real time in days after injection and localisation of BrdU among the stages of seminiferous epithelium expressed as relative time in percentage of the cycle. The slope indicates the speed of spermatogenesis.

Table 3
Comparative parameters in *Sorex araneus, Neomys fodien*s and *Crocidura russula* (± SE)

Parameters	*S. araneus*	*N. fodiens*	*C. russula*
Body mass (g)	10.9 ± 0.7[a]	16.8 ± 1.5*	14.2 ± 1.3[a]
Testes mass (g)	0.173 ± 0.021[a]	0.241 ± 0.041*	0.044 ± 0.004[a]
Relative testis mass (%)	1.60 ± 0.21[a]	1.4 ± 0.20*	0.30 ± 0.47[a]
Spermatogenic cycle length (days)	8.4 ± 0.26[a]	8.7 ± 0.14*	12.1 ± 0.60[a]
Total duration of spermatogenesis (days)	∼37.6[a]	∼39.1*	∼54.7[a]
Basal metabolic rate (BMR %)[b]	328[c]	183[d]	129[d]
Body temperature (°C)	∼39.0[e]	∼37.0[f]	∼35.5[e]
Multiple paternities	Yes[g]	Not known	No[h]

*This study.
[a] Parapanov et al. (2007).
[b] BMR %: BMR as a percentage of the value expected from the allometric relationship (McNab, 1988).
[c] Taylor (1998).
[d] Sparti (1990).
[e] Frey (1979).
[f] Vogel (1990).
[g] Searle (1990).
[h] Bouteiller and Perrin (2000).

Acknowledgments

We wish to express our gratitude to C. Wedekind for critical reading of the manuscript. We would also like to thank Anne-Marie Mehmeti for laboratory facilities. This work was supported by the University of Lausanne.

References

Adachi, Y., Kurohmaru, M., Hattori, S., Hayashi, Y., 1992. Spermatogenesis in the Watase's shrew, *Crocidura watasei*—a light electron microscopic study. Exp. Anim. 41 (3), 295–303.

Bedford, J.M., Cooper, G.W., Phillips, D.M., Dryden, G.L., 1994. Distinctive features of the gametes and reproductive tracts of the Asian musk shrew, *Suncus murinus*. Biol. Reprod. 50 (4), 820–834.

Bouteiller, C., Perrin, N., 2000. Individual reproductive success and effective population size in the greater white-toothed shrew *Crocidura russula*. Proc. R. Soc. Biol. Sci. Ser. B B267 (1444), 701–705.

Cantoni, D., 1993. Social and spatial organization of free-ranging shrews, *Sorex coronatus* and *Neomys fodiens* (Insectivora, Mammalia). Anim. Behav. 45 (5), 975–995.

Cantoni, D., Vogel, P., 1989. Social organisation and mating system of free-ranging, greater white-tooted shrews, *Crocidura russula*. Anim. Behav. 38, 205–214.

Crocock, C.A., Clark, J.R., 1976. Duration of spermatogenesis in the vole (*Microtus agrestis*) and bank vole (*Clethrionomys glareolus*). J. Reprod. Fertil. 47 (1), 133–135.

França, L.R., Becker-Silva, S.C., Chiarini-Garcia, H., 1999. The length of the cycle of seminiferous epithelium in goats (*Capra hircus*). Tissue Cell 31 (3), 274–280.

França, L.R., Ogawa, T., Avarbock, M.R., Brinster, R.L., Russell, L.D., 1998. Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol. Reprod. 59 (6), 1371–1377.

Frey, H., 1979. La température corporelle de Suncus etruscus (Soricidae, Insectivora) au cours de l'activité, du repos normothermique et de la torpeur. Rev. Suisse Zool. 86 (3), 653–662.

Garagna, S., Zuccotti, M., Searle, J., Redi, C.A., 1989. Histological description of the seminiferous epithelium cycle in the common shrew (*Sorex araneus* L.). Boll. Zool. 56, 299–303.

Harvey, P.H., Harcourt, A.H., 1984. Sperm competition, testes size and breeding systems in primates. In: Smith, R.L. (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York, pp. 589–600.

Kenagy, G.J., Trombulak, S.C., 1986. Size and function of mammalian testes in relation to body size. J. Mammal. 67, 1–22.

Kurohmaru, M., Kobayashi, H., Hattori, S., Nishida, T., Hayashi, Y., 1994. Spermatogenesis and ultrastructure of a peculiar acrosomal formation in the musk shrew, *Suncus murinus*. J. Anat. 185 (3), 503–509.

Leal, M.C., França, L.R., 2006. The seminiferous epithelium cycle length in the black tufted-ear marmoset (*Callithrix penicillata*) is similar to humans. Biol. Reprod. 74 (4), 616–624.

Leblond, C.P., Clermont, Y., 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann. N.Y. Acad. Sci. 55 (4), 548–573.

McNab, B.K., 1980. Food habits, energetics, and the population biology of mammals. Am. Nat. 116, 106–124.

McNab, B.K., 1988. Complications inherent in scaling the basal rate of metabolism in mammals. Q. Rev. Biol. 63, 25–54.

Meistrich, M.L., Eng, V.W.S., Loir, M., 1973. Temperature effects on the kinetics of spermatogenesis in the mouse. Cell Tissue Kinet. 6 (4), 379–393.

Møller, A.P., Birkhead, T.R., 1989. Copulation behaviour in mammals: evidence that sperm competition is widespread. Biol. J. Linn. Soc. 38, 119–131.

Oud, J.L., De Rooij, D.G., 1977. Spermatogenesis in the Chinese hamster. Anat. Rec. 187 (1), 113–124.

Parapanov, R., Nussle, S., Vogel, P., 2007. Cycle length of spermatogenesis in shrews (Mammalia: Soricidae) with high and low metabolic rates and different mating systems. Biol. Reprod. 76 (5), 833–840.

Parker, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45, 525–567.

Peirce, E.J., Breed, W.G., 2001. A comparative study of sperm production in two species of Australian arid zone rodents (*Pseudomys australis*, *Notomys alexis*) with marked differences in testis size. Reproduction 121 (2), 239–247.

Plöen, L., Ekwall, H., Afzelius, B.A., 1979. Spermiogenesis and the spermatozoa of the European common shrew (*Sorex araneus* L.). J. Ultrastruct. Res. 68 (2), 149–159.

Russell, L.D., Ettlin, R.A., Sinha-Hikim, A.P., Clegg, E.D., 1990. Mammalian spermatogenesis. In: Russell, L.D., Ettlin, R.A., Sinha-Hikim, A.P., Clegg, E.D. (Eds.), Histological and Histopathological Evaluation of the Testes. Cache River Press, Clearwater, FL, pp. 1–40.

Searle, J.B., 1990. Evidence for multiple paternity in the common shrew (*Sorex araneus*). J. Mammal. 71, 139–144.

Sharpe, R.M., 1994. Regulation of spermatogenesis. In: Knobil, E., Neil, J.D. (Eds.), The Physiology of Reproduction. Raven Press, New York, pp. 1363–1434.

Sparti, A., 1990. Comparative temperature regulation of African and European shrews. Comp. Biochem. Phys. 97 (A), 391–397.

Taylor, J.R.E., 1998. Evolution of Energetic Strategies in Shrews. In: Wojcik, J.M., Wolsan, M. (Eds), Evolution of Shrews. Mammal Research Institute, Polish Academy of Sciences, 309–338.

Vogel, P., 1990. Body temperature and fur quality in swimming Water-Shrews, *Neomys fodiens* (Mammalia, Insectivora). Z. Säugetierkunde. Verlag Paul Parey, Hambourg und Berlin, 55: 73–80.

Weinbauer, G.F., Schubert, J., Yeung, C.H., Rosiepen, G., Nieschlag, E., 1998. Gonadotrophin-releasing hormone antagonist arrests premeiotic germ cell proliferation but does not inhibit meiosis in the male monkey: a quantitative analysis using 5-bromodeoxyuridine and dual parameter flow cytometry. J. Endocrinol. 156 (1), 23–34.

Zeng, W., Avelar, G.F., Rathi, R., França, L.R., Dobrinski, I., 2006. The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J. Androl. 27 (4), 527–533.

CSIRO PUBLISHING

www.publish.csiro.au/journals/rfd *Reproduction, Fertility and Development*, 2008, **20**, 431–439

Relationships of basal metabolic rate, relative testis size and cycle length of spermatogenesis in shrews (Mammalia, Soricidae)

Roumen Parapanov[A,B], *Sébastien Nusslé*[A], *Jacques Hausser*[A] *and Peter Vogel*[A]

[A]Department of Ecology and Evolution, University of Lausanne, Lausanne CH-1015, Switzerland.
[B]Corresponding author. Email: roumen.parapanov@unil.ch

Abstract. The aim of the present study was to determinate the cycle length of spermatogenesis in three species of shrew, *Suncus murinus*, *Sorex coronatus* and *Sorex minutus*, and to assess the relative influence of variation in basal metabolic rate (BMR) and mating system (level of sperm competition) on the observed rate of spermatogenesis, including data of shrew species studied before (*Sorex araneus*, *Crocidura russula* and *Neomys fodiens*). The dynamics of sperm production were determined by tracing 5-bromodeoxyuridine in the DNA of germ cells. As a continuous scaling of mating systems is not evident, the level of sperm competition was evaluated by the significantly correlated relative testis size (RTS). The cycle durations estimated by linear regression were 14.3 days (RTS 0.3%) in *Suncus murinus*, 9.0 days (RTS 0.5%) in *Sorex coronatus* and 8.5 days (RTS 2.8%) in *Sorex minutus*. In regression and multiple regression analyses including all six studied species of shrew, cycle length was significantly correlated with BMR ($r^2 = 0.73$) and RTS ($r^2 = 0.77$). Sperm competition as an ultimate factor obviously leads to a reduction in the time of spermatogenesis in order to increase sperm production. BMR may act in the same way, independently or as a proximate factor, revealed by the covariation, but other factors (related to testes size and thus to mating system) may also be involved.

Introduction

The duration of the cycle of the seminiferous epithelium has been determined in several species of mammals. The values obtained are relatively constant within species, but vary notably between species. The cycle length may vary from 6.7 days in the bank vole (*Clethrionomys glareolus*; Crocock and Clark 1976) to 17 days in the Chinese hamster (*Cricetulus griseus*; Oud and De Rooij 1977). To date, experimental investigations have evidenced only two factors acting directly on cycle length of spermatogenesis, namely testicular temperature (Meistrich *et al*. 1973) and age (Van Haaster and De Rooij 1993). These factors operate at an intraspecific level. However, it is not clear which factors lead to interspecies variation in the cycle duration of spermatogenesis.

Intuitively, two life history factors might influence the cycle length, namely the basal metabolic rate (BMR) and the need to increase sperm production. Increased BMR may lead to accelerated tissue differentiation and consequently to a shorter cycle length (metabolic rate hypothesis). Alternatively, mating systems with high promiscuity resulting in multiple paternity may lead to selection for a higher sperm production, till now well known to be achieved by increasing relative testis size (RTS; Kenagy and Trombulak 1986) but it could be enhanced by a shorter cycle length as well (sperm competition hypothesis).

Shrews seem to be good candidates for a comparative examination of these hypotheses. Within the family of shrews, extreme forms exist for both factors. Initially, we compared two very divergent species (Parapanov *et al*. 2007), the monogamous greater white-toothed shrew (*Crocidura russula*), characterised by a small relative testis size and a practically normal basal metabolic rate, and the polygamous common shrew (*Sorex araneus*), where multiple paternity in litters is frequent, RTS is great and BMR is more than 300% of the size-expected value. The cycle length was, as expected, much longer in *Crocidura russula* than in *Sorex araneus*. However, these two species did not allow separation of the effects of metabolic rate and mating system; the variation could be influenced by one factor alone or by both factors combined. Moreover, if the evolutionary pressure is an optimal regulation of sperm production (ultimate factor), the mechanisms to achieve this regulation (proximate factors) could be either a variation in RTS or a variation in the metabolic rate and even, more directly, the possibly related body temperature (McNab 2002). In this case, a covariation between the factors is expected.

Ideally, for study purposes, one should find species with reversed character combinations; a monogamous shrew with high metabolic rate and a polygamous shrew with low metabolic rate. To our knowledge, this combination does not exist. Nonetheless, we may improve our understanding by increasing case studies of selected species deviating in BMR or mating systems from *Crocidura russula* and *Sorex araneus*. This was done for the water shrew (*Neomys fodiens*), with an intermediate BMR and a probably promiscuous mating system (Parapanov *et al*. 2008). With the three species in the present investigation, we enlarge the range of character combinations and may increase

 10.1071/RD07207 1031-3613/08/030431

insight into the possible mechanisms controlling the cycle length of spermatogenesis.

The first species, the Asian house shrew (*Suncus murinus*), is a tropical shrew of large body size that has a low metabolic rate typical for Crocidurinae. In relation to the metabolic rate hypothesis, we would expect a long cycle. From laboratory studies, it has been reported that females copulate frequently, sometimes even during gestation and lactation (Dryden *et al*. 1974; Clendenon and Rissman 1990), signalling the possibility of sperm competition that would lead to a shorter cycle. The second species, the Millet's shrew (*Sorex coronatus*) has a high metabolic rate (Genoud 1988) but has rather small testes (Turni 2003). According to the sperm competition hypothesis, this shrew should show a longer cycle, while the metabolic rate hypothesis would predict a short cycle. Finally, we included the pigmy shrew (*Sorex minutes*), a soricine shrew with a small body size of only 3–5 g with a very high metabolic rate. In this case, the shortest cycle is expected. The mating system in this species is unknown, but it is also not well-defined in most other shrew species. Instead of considering the mating system directly, we therefore used the relative testis size for all species, a characteristic that is easy to establish and is significantly correlated with the mating system (Kenagy and Trombulak 1986). Finally, single and multiple regression analyses including all six shrew species studied to date should permit us to evaluate the correlation between cycle length, BMR and RTS.

As in the previous studies, the dynamics of sperm production were determined by tracing 5-bromodeoxyuridine (BrdU) in the DNA of S-phase germ cells, which was incorporated into the nuclei of cells duplicating their DNA in preparation for mitosis or meiosis.

Materials and methods

Animals

Eleven adult male Asian musk shrews (*Suncus murinus*; 40–50 days of age) weighing 68.6 ± 5.7 g were used in the present study. The animals were born and raised in our colony at the University of Lausanne. The colony founders were provided in 2005 by Prof. K. Tsuji and were descended from a colony at the University of Nagoya, Japan. The colony was maintained on a light cycle of 12 h : 12 h light–dark photoperiod at a temperature of 25 ± 1°C. The shrews were caged individually, on natural soil and provided with food composed of minced meat with a vitamin supplement, mealworms, dried *Gammarus*, fish pellets and water *ad libitum*.

The soricine shrews were captured during the reproductive season, between April and June 2006, in the region of Lausanne, and Champtauroz, Switzerland. We used five adult male *Sorex coronatus*, weighing 10.2 ± 0.5 g and five adult male *Sorex minutus* weighing 5.3 ± 0.2 g. Soricine shrews attain maturity only in spring of their second calendar year; the wild animals used in this study were therefore all about one year old. They were kept in a dedicated building with a roof protecting them from direct rain, but with walls of wire mesh that exposed the animals to natural fluctuations of temperature and humidity according to the weather. The shrews were caged individually, on natural soil. They had *ad libitum* access to both water and food composed of minced meat with a vitamin supplement and mealworms.

Administration of BrdU

BrdU (BD Biosciences, PharMingen, Franklin Lakes, NJ, USA) was administered as a single intraperitoneal injection of 50 mg kg^{-1} for each male. BrdU administration was well-tolerated and no negative effects were observed in the three species.

Suncus murinus males were killed by halothane overdose at the following times: three males at 3 h, three at 8 days 3 h, two at 16 days 3 h and three at 23 days 3 h after administration of BrdU. *Sorex coronatus* males were killed at the following time points: two at 3 h, one at 8 days 3 h and two at 16 days 3 h after administration of BrdU. *Sorex minutus* males were killed at the same times points: two at 3 h, two at 8 days 3 h and one at 16 days 3 h after injection of BrdU.

The testes were removed, weighed and fixed in a 4% solution of paraformaldehyde for 3 days, dehydrated in a graded series of ethanol and embedded in Paraplast. Histological sections (3 μm) of the testes were dewaxed and rehydrated. BrdU was localised using a ZYMED BrdU Staining Kit provided by Invitrogen, Carlsbad, CA, USA. Endogenous peroxidase was inactivated in 3% (v/v) H_2O_2 in methanol for 10 min at room temperature. To expose BrdU for immunohistochemical localisation, DNA was denatured in 0.17% (v/v) trypsin solution for 10 min at 37°C in a moist chamber. Incubation with a biotinylated anti-BrdU antibody was performed for 1 h at room temperature. Sections were then incubated with streptavidin–horse radish peroxidase for 10 min at room temperature. Diaminobenzidine substrate solution was added to cover the tissue sections and they were incubated for 5 min or until the colour intensity had developed. Periodic acid Schiff (PAS)–haematoxylin staining was performed for identification of spermatogenic stages. Incubation with 1% periodic acid (for 10 min at room temperature) and staining with Schiff's reagent (for 30 min at room temperature) was followed by incubation with Mayer's haematoxylin solution (5 min at room temperature). Histological preparations were observed under an Axiophot microscope (Zeiss, Oberkochen, Germany).

Stage frequencies, staining frequencies and duration of the seminiferous epithelium cycle

In *Suncus murinus* we used the 13 stages of seminiferous epithelium already described by Kurohmaru *et al.* (1994) (see Table 1). In *Sorex coronatus* and *Sorex minutus* we identified 10 stages. The characteristics of stages of the seminiferous epithelium in the two *Sorex* species correspond to the situation found in *Sorex araneus* (Plöen *et al*. 1979; Garagna *et al*. 1989).

The stage determination is based on the spermatid morphology method and the number of stages has no influence on the estimation of the total cycle length.

The relative duration of each stage of the cycle in the two soricine shrews was determined from the testes sections counterstained with PAS–haematoxylin. We counted only round cross-sections of the seminiferous tubules.

The stage frequencies, expressed as percentages, correspond to the relative duration of the stages of the cycle. We counted 200–300 tubular cross-sections per testis. A total of 1500 tubules from five *Sorex coronatus* and 1400 from five *Sorex minutus*

Table 1. Comparative parameters in six species of shrews (mean ± s.e.m.)

Parameter	*S. murinus*	*S. coronatus*	*S. minutus*	*S. araneus*	*N. fodiens*	*C. russula*
Body mass (g)	68.6 ± 5.69	10.2 ± 0.5	5.2 ± 0.2	10.9 ± 0.7[B]	16.8 ± 1.5[C]	14.2 ± 1.3[B]
Testes mass (g)	0.169 ± 0.028	0.053 ± 0.007	0.148 ± 0.016	0.173 ± 0.021[B]	0.241 ± 0.041[C]	0.044 ± 0.004[B]
Relative testis mass (%)	0.3 ± 0.03	0.5 ± 0.07	2.8 ± 0.30	1.6 ± 0.21[B]	1.4 ± 0.20[C]	0.3 ± 0.47[B]
Spermatogenic cycle length (days)	14.3 ± 0.12	9.0 ± 0.19	8.5 ± 0.15	8.4 ± 0.26[B]	8.7 ± 0.14[C]	12.1 ± 0.60[B]
Total duration of spermatogenesis (days)	~64.4	~40.4	~38.3	~37.6[B]	~39.1[C]	~54.7[B]
Basal metabolic rate (BMR %)[A]	153[D]	322[E]	366[E]	328[F]	183[G]	129[G]
BMR (mL O_2 g^{-1} h^{-1})	1.7[D]	5.7[E]	8.6[E]	6.1[F]	2.9[G]	2.2[G]
Body temperature (°C)	~35.3[H]	~37.6[E]	~38.5[E]	~39.0[E]	~37.0[I]	~35.5[H]
Multiple paternity	Not known	Not known	Not known	Yes[J]	Not known	No[K]

[A]BMR % = BMR as a percentage of the value expected from the allometric relationship (McNab 1988); BMR = mass-specific basal metabolic rate in mL O_2 consumption per g, per hour.
[B]Parapanov *et al*. (2007).
[C]Parapanov *et al*. (2008).
[D]Dryden *et al*. (1974).
[E]Sparti and Genoud (1989).
[F]Taylor (1998).
[G]Sparti (1990).
[H]Frey (1979).
[I]Vogel (1990).
[J]Searle (1990).
[K]Bouteiller and Perrin (2000).

were scored. The estimation of staining frequency was based on the percentage of tubules of a given stage containing the most-advanced germ cells labelled by BrdU. The stage and staining frequencies were calculated according to the methods used previously by Parapanov *et al*. (2007). The duration of the cycle of seminiferous epithelium was estimated by the linear regression method described by Parapanov *et al*. (2007).

Statistical analysis

For interspecific analyses, data on cycle length of spermatogenesis and RTS (testes/body percent) were used from all our studies (Parapanov *et al*. 2007, 2008). Basal metabolic rate data were collected from the literature (Nagel 1980, 1985; Sparti 1990; Taylor 1998) and always presented and used as 'mass-specific basal metabolic rate' (mL O_2 g^{-1} h^{-1}). After logarithmic transformation of all factors, a multiple linear regression was performed with cycle length (log days) as the response variable, and BMR (log mL O_2 g^{-1} h^{-1}) and RTS (log % of body mass) as independent variables. Analyses of variance were then performed, and, as BMR and RTS were correlated, both factors were permutated in order to disentangle the different effects. We also used linear regression to test the relationship between cycle length and BMR and RTS independently.

All data are presented as mean ± s.e.m. and all analyses were performed with R software (R Development Core Team 2007). The significance level was considered to be 0.05, but P values between 0.1 and 0.05 were regarded as tendencies because variation is high when dealing with living organisms and low sample size. We used a Bonferroni correction when multiple comparisons were performed.

Ethical considerations

This project was carried out under authorisation of the State Commission for Animal Experiments of Canton de Vaud (1707.1 VD). The number of samples of wild shrews was kept as low as possible because all species of shrew are protected in Switzerland.

Results

Testicular weight

Measurements such as body mass, testis mass and relative testis size are given in Table 1. The absolute testis mass of the large *Suncus murinus* (0.169 ± 0.026 g) was not significantly different from that of the tiny *Sorex minutus* (0.148 ± 0.016 g) (Welch modified t-test for non-equal variance; $t = -1.85$, d.f. = 12.64, $P = 0.09$), but was significantly different from the testis mass of *Sorex coronatus* (0.053 ± 0.007 g) (Welch modified t-test for non-equal variance; $t = -13.20$, d.f. = 12.17, $P < 0.0001$). In contrast, the relative testis size expressed as a percentage of body mass was significantly higher in *Sorex minutus* (2.8%) than in *Sorex coronatus* (0.5%) (Welch modified t-test for non-equal variance; $t = 16.52$, d.f. = 4.48, $P < 0.0001$), and *Suncus murinus* (0.3%) (Welch modified t-test for non-equal variance; $t = 18.99$, d.f. = 4.04, $P < 0.0001$).

BrdU immunohistochemistry

In *Suncus murinus*, the most-advanced BrdU-labelled cells at 3 h were leptotene spermatocytes in Stage VI. At 8 days 3 h, the label was detected in the pachytene spermatocytes in Stage I. At 16 days 3 h, the label had progressed to the pachytene spermatocytes

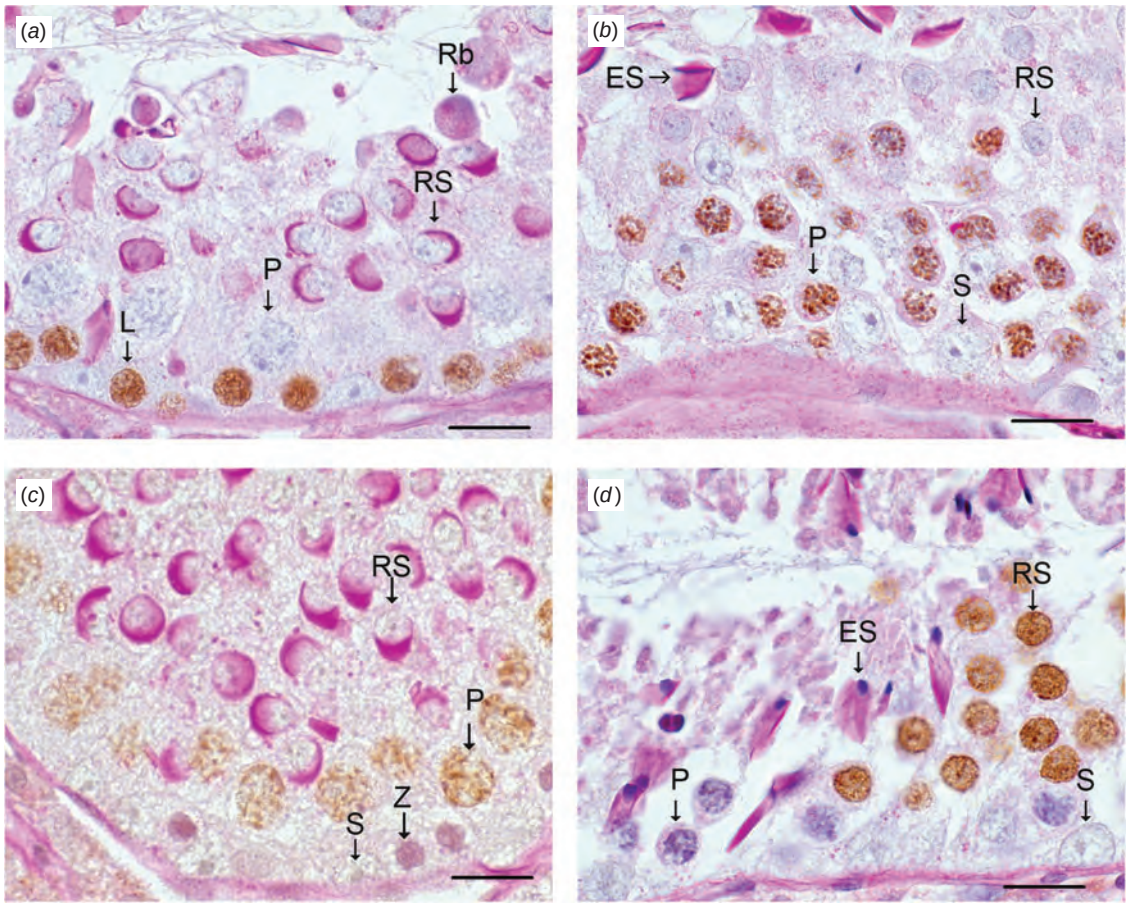


Fig. 1. Most-advanced labelled germ cells in *Suncus murinus* observed at different time periods after BrdU injection. Three hours after BrdU injection, leptotene spermatocytes (L) at Stage VI (*a*). Eight days and 3 h after injection, pachytene spermatocytes (P) at Stage I (*b*). Sixteen days and 3 h after injection, pachytene spermatocytes at Stage VII (*c*). Twenty-three days and 3 h after injection, round spermatids (RS) at Stage I (*d*). B spermatogonia (B), preleptotene spermatocytes (PL), elongated spermatids (ES), Sertoli cells (S), residual bodies (Rb), zigotene spermatocytes (Z). Scale bars = 20 μm.

in Stage VII. At 23 days 3 h after injection, BrdU was localised in spermatids Step 1 in Stage I (Fig. 1).

In *Sorex coronatus* and *Sorex minutus*, 3 h after BrdU injection, the most advanced labelled cells were the preleptotene spermatocytes in Stage VI. At 8 days 3 h, the most advanced cells containing BrdU were pachytene spermatocytes in the same Stage VI. At 16 days 3 h, the most advanced BrdU-labelled cells were round spermatids in Stage V (Fig. 2).

Stage frequency, staining frequency and duration of the seminiferous epithelium cycle

The mean percentages of the stage frequencies for *Suncus murinus*, *Sorex coronatus* and *Sorex minutus* are shown in Table 2. The staining frequencies (Parapanov *et al*. 2007) are given in Table 3. The cycle durations obtained by linear regression were 14.3 ± 0.1 days ($r^2 = 0.999$) in *Suncus murinus*, 9.0 ± 0.2 days ($r^2 = 0.998$) in *Sorex coronatus* and 8.5 ± 0.5 days ($r^2 = 0.999$) in *Sorex minutus* (Fig. 3). The total duration of spermatogenesis based on 4.5 cycles (Russell *et al*. 1990) was calculated to be 64.4 days in *Suncus murinus*, 40.4 days in *Sorex coronatus* and 38.3 days in *Sorex minutus*.

Relationships of relative testis size, metabolic rate and cycle length of spermatogenesis

We found an inverse correlation between the cycle length of spermatogenesis and BMR ($n = 6$, $r^2 = 0.73$, $P = 0.03$, Fig. 4*a*) and between the cycle length of spermatogenesis and RTS ($n = 6$, $r^2 = 0.77$, $P = 0.02$, Fig. 4*b*). Multiple regression analysis showed that both BMR and RTS were significantly associated with the cycle length of spermatogenesis (Table 4).

Discussion

Cycle length, metabolic rate and relative testis size in Suncus murinus, Sorex coronatus *and* Sorex minutus

Previous studies comparing two very different shrews, *Crocidura russula* and *Sorex araneus*, suggested that metabolic rate, and

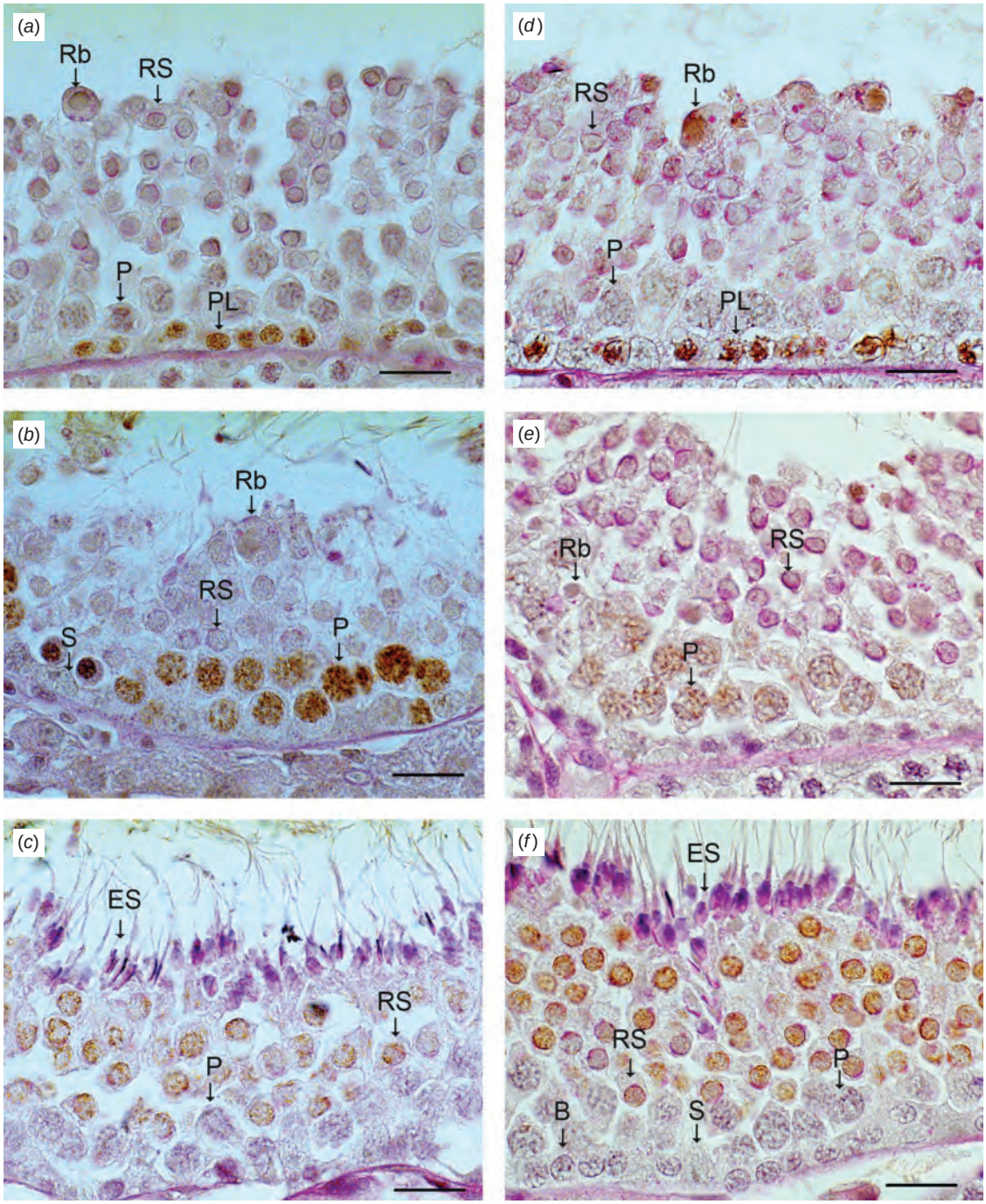


Fig. 2. Most-advanced labelled germ cells in *Sorex coronatus* observed at different time periods after BrdU injection. Three hours after BrdU injection, preleptotene spermatocytes (PL) at stage VI (*a*). Eight days and 3 h after injection, pachytene spermatocytes (P) at Stage VI (*b*). Sixteen days and 3 h after injection, round spermatids (RS) at Stage V (*c*). Most-advanced labelled germ cells in *Sorex minutus* observed at different time periods after BrdU injection. Three hours after BrdU injection, preleptotene spermatocytes at Stage VI (*d*). Eight days and 3 h after injection, pachytene spermatocytes at Stage VI (*e*). Sixteen days and 3 h after injection, round spermatids at Stage V (*f*). B spermatogonia (B), elongated spermatids (ES), Sertoli cells (S), residual bodies (Rb). Scale bars = 20 μm.

Table 2. Stage frequencies of the seminiferous epithelium cycle

The duration (in days) is given for information and was calculated from the cycle length inferred from linear regression

	Stage												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Suncus murinus[A]													
Relative duration of stage (%)	5.1	5.9	10.1	8.8	12.5	11.5	10.6	7.9	6.0	4.8	8.9	3.1	4.8
Sorex coronatus													
Relative duration of stage (%)	13.8 ± 3.3	7.8 ± 1.3	6.5 ± 2.5	7.3 ± 2.2	15.6 ± 4.3	20.1 ± 2.8	9.0 ± 1.5	4.8 ± 1.2	12.4 ± 3.8	2.6 ± 0.5			
Days	1.2	0.7	0.6	0.7	1.4	1.8	0.8	0.4	1.1	0.2			
Sorex minutus													
Relative duration of stage (%)	16.2 ± 3.1	8.1 ± 2.9	10.4 ± 3.3	13.4 ± 2.7	18.4 ± 3.0	9.8 ± 4.2	7.0 ± 1.6	5.8 ± 1.5	8.4 ± 3.4	2.5 ± 0.3			
Days	1.4	0.7	0.9	1.2	1.6	0.8	0.6	0.5	0.7	0.2			

[A]For *S. murinus* we used the stage frequencies calculated by Kurohmaru *et al*. (1994).

relative testis size (resulting from natural selection for sperm competition), or both, could have an important influence on the spermatogenesis cycle length (Parapanov *et al*. 2007). According to McNab (1980) a high metabolic rate could increase the rate of tissue production and differentiation. Therefore, we hypothesised that high metabolic rate could accelerate sperm production. Moreover, *Sorex araneus* is a classic example of a multiple paternity shrew species (Searle 1990; Stockley *et al*. 1993), and it is therefore a good candidate for a species showing sperm competition. As expected, *Sorex araneus* also showed large relative testis size in agreement with data from Harvey and Harcourt (1984) and Kenagy and Trombulak (1986). Shortening the spermatogenesis cycle length in order to increase the rate of sperm production would appear to be a valuable mechanism for increasing sperm production per unit of testis volume.

In the present study, we aimed to investigate species with particular characteristics: *Suncus murinus* with a relatively low metabolic rate (Dryden *et al*. 1974), *Sorex coronatus* with a smaller testis size (Turni 2003) and *Sorex minutus* with a very high metabolic rate (Sparti and Genoud 1989), to assess whether cycle length varied according to our predictions, and to assess the relationships of metabolic rate and relative testis size with cycle length.

Among the species we studied *Suncus murinus* displayed the longest cycle length of 14.3 days. This finding favours the metabolic rate hypothesis, as in this largest crocidurine shrew studied to date, the relative basal metabolic rate (per gram) is much lower than in *Crocidura russula* (Table 1), which has a shorter cycle length of 12.1 days. In the context of sperm competition our results (Table 1) showed low RTS in *Suncus murinus* (0.3%), which is similar to that of the monogamous *Crocidura russula*. Although laboratory reports characterised this species as a promiscuous shrew (S. Siniza, pers. comm.), the RTS correlation with mating systems (Harvey and Harcourt 1984; Kenagy and Trombulak 1986; Møller 1988) suggests a monogamous species.

Sorex coronatus has a similar body size to *Sorex araneus*, but the cycle length of 9.0 days is significantly longer and the spermatogenesis is markedly slower than in the latter species. Since the energy requirements of these two species are similar, the metabolic rate hypothesis cannot be evoked. In contrast, the question of the influence of mating system via RTS arises since the RTS of 0.5% in *Sorex coronatus* is relatively low, suggesting a mating system characterised by low promiscuity. This was also proposed by Turni (2003) citing the results of Cantoni (1993) whose radio-telemetric studies suggest mate-guarding behaviour in this species.

Sorex minutus, the smallest soricine shrew from our region (adult weight ~5 g), is characterised by the highest basal metabolic rate (Sparti and Genoud 1989). According to the metabolic rate hypothesis, with a BMR value of 366% of the mammalian mean value expected from body mass, the cycle length should be shorter than in *Sorex araneus* with its BMR of 328%. However, with a cycle length of 8.5 days, its rate of spermatogenesis is slightly slower. From its high RTS of 2.8%, this species should be extremely promiscuous (Kenagy and Trombulak 1986), even more so than *Sorex araneus* with its RTS of

Table 3. Staining frequencies of seminiferous epithelium

Time after injection	Number of animal	Stage VI		Stage VII		Stage I	
		Staining fr. (%)	Stage fr. (%)	Staining fr. (%)	Stage fr. (%)	Staining fr. (%)	Stage fr. (%)
Suncus murinus							
3 h[A]	14	37.5	11.5				
	24	55.4	11.5				
	28	36.5	11.5				
8 d 3 h[B]	13					41.3	5.1
	38					66.2	5.1
	40					71.7	5.1
16 d 3 h[B]	50			58.8	10.6		
	54			65.5	10.6		
23 d 3 h[C]	35					69.3	5.1
	51					76.8	5.1
	52					40.7	5.1

Time after injection	Number of animal	Stage VI		Stage V	
		Staining fr. (%)	Stage fr. (%)	Staining fr. (%)	Stage fr. (%)
Sorex coronatus					
3 h[A]	614	55.4	20.1		
	619	72.3	20.1		
8 d 3 h[B]	616	40.2	20.1		
16 d 3 h[C]	615			29.4	15.6
	618			60.5	15.6
Sorex minutus					
3 h[A]	1	56.6	9.8		
	613	51.5	9.8		
	2	29.8	9.8		
	610	14.7	9.8		
16 d 3 h[C]	612			49.5	18.4

[A]Most-advanced BrdU-labelled cells were preptotene-leptotene spermatocytes.
[B]Most-advanced BrdU-labelled cells were pachytene spermatocytes.
[C]Most-advanced BrdU-labelled cells were round spermatids.

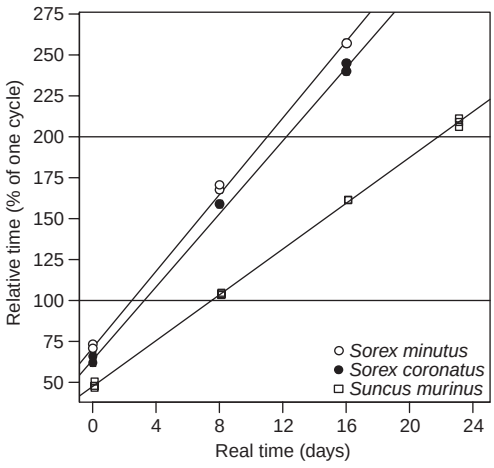


Fig. 3. Diagram showing the relationships between elapsed real time in days after injection and localisation of BrdU among the stages of seminiferous epithelium expressed as relative time in percentage of the cycle. The slopes indicate the speed of spermatogenesis. Open circles represent *Sorex minutus*, filled circles represent *Sorex coronatus* and open squares represents *Suncus murinus*.

1.6%. Therefore, a direct effect of testis size on cycle length is not apparent in this instance.

Correlation analyses of cycle length, basal metabolic rate and relative testis size in shrews

The expansion of the range of species examined in the present study allowed the investigation of possible correlations between cycle length and basal metabolic rate (metabolic rate hypothesis) as well as cycle length and relative testis size (sperm competition hypothesis). In agreement with the sperm competition theory, the latter would be involved with the mating system (Harvey and Harcourt 1984; Kenagy and Trombulak 1986; Møller and Birkhead 1989) together with other adaptations including sperm maturation (Jones 1999) and sperm storage in epididymis and vas deferens (Suzuki and Racey 1984; Bedford *et al*. 1994).

Analysed separately, cycle length as a response variable on mass-specific basal metabolic rate as an independent variable showed a significant correlation ($r^2 = 0.73$, $P = 0.03$). Cycle length as a response variable on relative testis size as an independent variable also showed a significant correlation ($r^2 = 0.77$, $P = 0.02$). As the analyses of variance are highly significant, we conclude that cycle length of spermatogenesis may be influenced by both factors, metabolic rate and relative testis size

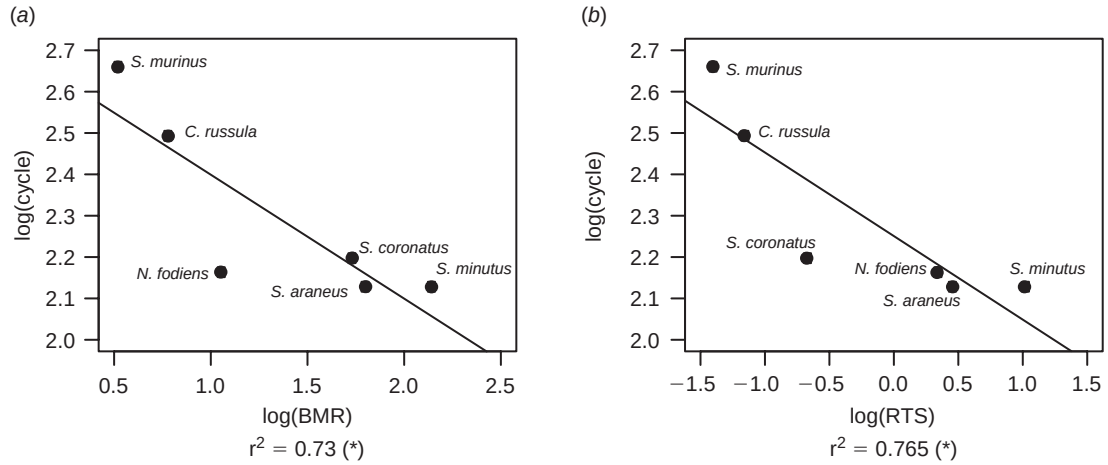


Fig. 4. Diagram showing the correlation between (*a*) the cycle length of spermatogenesis and BMR ($n = 6$, $r^2 = 0.73$, $P = 0.03$), and (*b*) the cycle length of spermatogenesis and RTS ($n = 6$, $r^2 = 0.77$, $P = 0.02$).

Table 4. ANOVA table from multiple regression analysis
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS (not significant at $P > 0.05$)

	Estimate	Std error	*t*-value	Pr (>|*t*|)
(Intercept)	2.33873	0.01992	117.423	7.25e-05***
log(BMR)	−0.10797	0.01249	−8.644	0.01312*
log(RTS)	−0.36214	0.01520	−23.827	0.00176**
log(BMR) : log(RTS)	0.17905	0.00946	18.928	0.00278**

which is a consequence of sperm competition. Our results are in agreement with the suggestion that the higher rate of sperm production in birds is probably related to the higher metabolic rate (Jones 2002). Moreover, it was evidenced that in Japanese quail (*Coturnix coturnix japonica*), a higher relative testis size of 2.6% (Clulow and Jones 1982) is correlated with a short cycle length of spermatogenesis (Lin *et al*. 1990).

As reported in the introduction, sperm competition seems to be the ultimate factor, selecting for an up or downregulation of sperm production. Basal metabolic rate could be a proximate factor especially via the related body temperature (McNab 2002) and the testicular temperature which may have a direct effect on cycle length (Meistrich *et al*. 1973). As testicular temperature is different from the body temperature (Carrick and Setchell 1977; Bedford *et al*. 1982) and unfortunately not known for most shrews, we cannot introduce this factor into our model. Basically, deviations of the basal metabolic rate in mammals from the size-expected value are generally considered as a physiological adaptation to different environmental conditions. Climatic adaptations in the context of thermoregulation (Vogel 1976), adaptations related to food regime (McNab 1980), or adaptation to regulate the reproductive output in females (McNab 1980) may have a larger impact than sperm competition. Another proximate factor obviously participates in the mediation of the cycle length as shown by the case of *Neomys fodiens* (Parapanov *et al*. 2008), with a rather low BMR, a high RTS value and a short cycle (Fig. 4), and by the case of *Sorex coronatus*, with a high BMR, a low RTS value and a longer cycle (Fig. 4). This unknown factor could be directly related to testis size and thus to mating system.

Finally, the effects of taxonomic relationships may also play an important role. An analysis of the various factors influencing cycle length in a larger range of mammal species using phylogenetic contrasts could help to unravel the question of the main determinants of spermatogenesis cycle length.

References

Bedford, J. M., Berrios, M., and Dryden, G. L. (1982). Biology of the scrotum. IV. Testis location and temperature sensitivity. *J. Exp. Zool.* **224**, 379–388. doi:10.1002/JEZ.1402240311

Bedford, J. M., Cooper, G. W., Phillips, D. M., and Dryden, G. L. (1994). Distinctive features of the gametes and reproductive tracts of the Asian musk shrew, *Suncus murinus*. *Biol. Reprod.* **50**, 820–834. doi:10.1095/BIOLREPROD50.4.820

Bouteiller, C., and Perrin, N. (2000). Individual reproductive success and effective population size in the greater white-toothed shrew *Crocidura russula*. *Proc. R. Soc. Biol. Sci. Ser. B* **267**, 701–705. doi:10.1098/RSPB.2000.1059

Cantoni, D. (1993). Social and spatial organization of free-ranging shrews, *Sorex coronatus* and *Neomys fodiens* (Insectivora, Mammalia). *Anim. Behav.* **45**, 975–995. doi:10.1006/ANBE.1993.1116

Carrick, F. N., and Setchell, B. P. (1977). The evolution of the scrotum. In 'Reproduction and Evolution'. (Eds J. H. Calaby and T. H. Tyndale-Biscoe.) pp. 165–170. (Australian Academy of Science: Canberra.)

Clendenon, A. L., and Rissman, E. F. (1990). Prolonged copulatory behaviour facilitates pregnancy in the Musk Shrew. *Phys. Behav.* **47**, 831–835. doi:10.1016/0031-9384(90)90005-O

Clulow, J., and Jones, R. C. (1982). Production, transport, maturation, storage and survival of spermatozoa in the male Japanese quail, *Coturnix coturnix*. *J. Reprod. Fertil.* **64**, 259–266.

Crocock, C. A., and Clark, J. R. (1976). Duration of spermatogenesis in the vole (*Microtus agrestis*) and bank vole (*Clethrionomys glareolus*). *J. Reprod. Fertil.* **47**, 133–135.

Dryden, G. L., Gebczynski, M., and Douglas, E. L. (1974). Oxygen consumption by nursling and adult musk shrews. *Acta Theriol. (Warsz.)* **19**, 453–461.

Frey, H. (1979). La température corporelle de *Suncus etruscus* (Soricidae, Insectivora) au cours de l'activité, du repos normothermique et de la torpeur. *Rev. Suisse Zool.* **86**, 653–662.

Garagna, S., Zuccotti, M., Searle, J., and Redi, C. A. (1989). Histological description of the seminiferous epithelium cycle in the common shrew (*Sorex araneus* L.). *Boll. Zool.* **56**, 299–303.

Genoud, M. (1988). Energetics strategies of shrews: ecological constraints and evolutionary implications. *Mammal Rev.* **18**, 173–193.

Harvey, P. H., and Harcourt, A. H. (1984). Sperm competition, testes size and breeding systems in primates. In 'Sperm competition and the Evolution of Animal Mating Systems'. (Ed. R. L. Smith.) pp. 589–600. (Academic Press: New York.)

Jones, R. C. (1999). To store or mature spermatozoa? The primary role of the epididymis. *Int. J. Androl.* **22**, 57–67. doi:10.1046/J.1365-2605.1999.00151.X

Jones, R. C. (2002). Evolution of epididymis. In 'The Epididymis: From Molecules to Clinical Practice – A Comprehensive Survey of the Ducts, the Epididymis and the Vas Deferens'. (Eds B. Robaire and B. T. Hinton.) pp. 11–33. (Kluwer Academic/Plenum Publishers: New York.)

Kenagy, G. J., and Trombulak, S. C. (1986). Size and function of mammalian testes in relation to body size. *J. Mammal.* **67**, 1–22. doi:10.2307/1380997

Kurohmaru, M., Kobayashi, H., Hattori, S., Nishida, T., and Hayashi, Y. (1994). Spermatogenesis and ultrastructure of a peculiar acrosomal formation in the musk shrew, *Suncus murinus*. *J. Anat.* **185**, 503–509.

Lin, M., Jones, R. C., and Blackshaw, A. W. (1990). The cycle of the seminiferous epithelium in the Japanese quail (*Coturnix coturnix japonica*) and estimation of its duration. *J. Reprod. Fertil.* **88**, 481–490.

McNab, B. K. (1980). Food habits, energetics, and the population biology of mammals. *Am. Nat.* **116**, 106–124. doi:10.1086/283614

McNab, B. K. (1988). Complications inherent in scaling the basal rate of metabolism in mammals. *Q. Rev. Biol.* **63**, 25–54. doi:10.1086/415715

McNab, B. K. (2002). 'The Physiological Ecology of Vertebrates.' (Cornell University Press: Ithaca, NY.)

Meistrich, M. L., Eng, V. W. S., and Loir, M. (1973). Temperature effects on the kinetics of spermatogenesis in the mouse. *Cell Tissue Kinet.* **6**, 379–393.

Møller, A. P. (1988). Ejaculate quality, testes size and sperm competition in primates. *J. Hum. Evol.* **17**, 479–488. doi:10.1016/0047-2484(88)90037-1

Møller, A. P., and Birkhead, T. R. (1989). Copulation behaviour in mammals: evidence that sperm competition is widespread. *Biol. J. Linn. Soc.* **38**, 119–131. doi:10.1111/J.1095-8312.1989.TB01569.X

Nagel, A. (1980). Sauerstoffverbrauch,Temperaturregulation und Herzfrequenz der europäischen Spitzmäuse (Soricidae, Mammalia). Ph.D. Thesis, Eberhard-Karls-Univerisität, Tübingen, FRG.

Nagel, A. (1985). Sauerstoffverbrauch,Temperaturregulation und Herzfrequenz bei europäischen Spitzmäuse (Soricidae). *Z. Säugetierkunde*. **50**, 249–266.

Oud, J. L., and De Rooij, D. G. (1977). Spermatogenesis in the Chinese hamster. *Anat. Rec.* **187**, 113–124. doi:10.1002/AR.1091870109

Parapanov, R., Nussle, S., and Vogel, P. (2007). Cycle length of spermatogenesis in shrews (Mammalia: Soricidae) with high and low metabolic rates and different mating systems. *Biol. Reprod.* **76**, 833–840. doi:10.1095/BIOLREPROD.106.058073

Parapanov, R., Nussle, S., Hausser, J., and Vogel, P. (2008). Histological description of seminiferous epithelium and cycle length of spermatogenesis in the water shrew *Neomys fodiens* (Mammalia: Soricidae). *Anim. Reprod. Sci.* in press.

Plöen, L., Ekwall, H., and Afzelius, B. A. (1979). Spermiogenesis and the spermatozoa of the European common shrew (*Sorex araneus* L.). *J. Ultrastruct. Res.* **68**, 149–159. doi:10.1016/S0022-5320(79)90150-3

R Development Core Team (2007). 'R: A language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna, Austria.)

Russell, L. D., Ettlin, R. A., Sinha-Hikim, A. P., and Clegg, E. D. (1990). Mammalian spermatogenesis. In 'Histological and Histophatological Evaluation of the Testes'. (Eds L. D. Russell, R. A. Ettlin, A. P. Sinha-Hikim and E. D. Clegg.) pp. 1–40. (Cache River Press: Clearwater, FL.)

Searle, J. B. (1990). Evidence for multiple paternity in the common shrew (*Sorex araneus*). *J. Mammal.* **71**, 139–144. doi:10.2307/1382159

Sparti, A. (1990). Comparative temperature regulation of African and European shrews. *Comp. Biochem. Phys.* **97**, 391–397. doi:10.1016/0300-9629(90)90629-7

Sparti, A., and Genoud, M. (1989). Basal rate of metabolism and temperature regulation in *Sorex coronatus* and *Sorex minutus* (Soricidae:Mammalia). *Comp. Biochem. Phys. Series A* **92**, 359–363. doi:10.1016/0300-9629(89)90576-8

Stockley, P., Searle, J. B., Macdonald, D. W., and Jones, C. S. (1993). Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proc. R. Soc. Lond. B. Biol. Sci.* **254**, 173–179. doi:10.1098/RSPB.1993.0143

Suzuki, F., and Racey, P. A. (1984). Light and electron microscopical observations on the male excurrent duct system of the common shrew (*Sorex araneus*). *J. Reprod. Fertil.* **70**, 419–428.

Taylor, J. R. E. (1998). Evolution of Shrews. In 'Evolution of Energetic Strategies in Shrews'. (Eds J. M. Wojcik and M. Wolsan.) pp. 309–338. (Mammal Research Institute, Polish Academy of Sciences: Bielowieza.)

Turni, H. (2003). Zur Ökologie und Reproduktionsbiologie der Geschwisterarten Waldspitzmaus (*Sorex araneus* L. 1758) und Schabrackenspitzmaus (*Sorex coronatus* Millet 1828). Ph.D. Thesis, Eberhard-Karls-Universität Tübingen, Germany.

Van Haaster, L. H., and De Rooij, D. G. (1993). Spermatogenesis is accelerated in the immature Djungarian and Chinese hamster and rat. *Biol. Reprod.* **49**, 1229–1235. doi:10.1095/BIOLREPROD49.6.1229

Vogel, P. (1976). Energy consumption of European and African shrews. *Acta Theriol. (Warsz.)* **21**, 195–206.

Vogel, P. (1990). Body temperature and fur quality in swimming Water-Shrews, *Neomys fodiens* (Mammalia, Insectivora). *Z. Säugetierkunde. Verlag Paul Parey Hambourg und Berlin* **55**, 73–80.

Manuscript received 16 November 2007, accepted 20 January 2008

Animal Reproduction Science 114 (2009) 269–278

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Testis size, sperm characteristics and testosterone concentrations in four species of shrews (Mammalia, Soricidae)

R.N. Parapanov[a,*], S. Nusslé[a], M. Crausaz[b], A. Senn[b], J. Hausser[a], P. Vogel[a]

[a] *Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland*
[b] *Foundation F.A.B.E.R., rue de la Vigie 5, 1003 Lausanne, Switzerland*

ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form 8 September 2008
Accepted 23 September 2008
Available online 1 October 2008

Keywords:
Sperm competition
Testis size
Sperm number
Sperm motility
Sperm length
Testosterone concentration

ABSTRACT

The aim of this study was to establish and compare the sperm characteristics in four shrew species in the context of the sperm competition hypothesis. As expected, the large relative testis size in promiscuous species was associated with a high number of cauda epididymal spermatozoa and a high concentration of circulating testosterone. In addition, in *Sorex* and *Neomys*, species with high intensity of sperm competition, the spermatozoa stored in cauda epididymis were characterized by high percentage of progressive motility whereas in *Crocidura* and *Suncus*, the cauda epididymal spermatozoa were motile but with very low percentage of progressive motility. This capability is achieved only following the passage through the vas gland, a specialized region for sperm storage located along the vas deferens in these shrew species. The hypothesis that sperm competition is positively correlated with spermatozoa length could not be confirmed. In *Crocidura* and *Suncus*, the total sperm length is increased by the large sperm head due to a big acrosome. This trait, specific to the subfamily Crocidurinae, may results from a selective pressure independent of the context of sperm competition, related to a specific, but as yet unclear role, for the acrosome during the fertilization.

1. Introduction

The efficiency of spermatogenesis is a key to reproductive success in the male. Male fertility is characterized by many important contributing factors. One of the most important is the number of

* Corresponding author.
E-mail address: roumen.parapanov@unil.ch (R.N. Parapanov).

0378-4320/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2008.09.013

spermatozoa available for fertilizing oocytes. According to Parker (1970), sperm production may be interpreted in relation to sperm competition, i.e. the competition between the spermatozoa of different males within a female. The selective pressure associated with multiple mating and competition among the spermatozoa within the female genital tract may lead to the evolution of larger testes and higher sperm numbers, a statement already supported by several studies (Harcourt et al., 1981; Parker, 1982; Harvey and Harcourt, 1984; Kenagy and Trombulak, 1986; Møller and Birkhead, 1989; Gomendio and Roldan, 1993). Moreover, a relationship between mating systems, circulating testosterone and sexual behaviour in male mammals has been also reported (Dixson and Anderson, 2004). Ramm et al. (2005) demonstrated that in rodents there was a positive association between testis size and multiple paternities and between sperm competition level and the size of some accessory sex glands. In addition, Gomendio et al. (2006) showed in four mice species that sperm competition is associated with enhanced functional capacity of spermatozoa. Sperm competition can lead not only to increased sperm production but also to improvements in some traits of ejaculate quality, including sperm motility (Møller and Birkhead, 1989; Birkhead et al., 1999; Aslam et al., 2002; Gage et al., 2004), sperm viability as the proportion of live sperm (Hunter and Birkhead, 2002), and sperm morphology (Stockley et al., 1997; Calhim et al., 2007). In contrast, monogamous species without sperm competition commonly have smaller testes and in the absence of selective pressure, sperm mobility and ejaculate quality may be lower.

Reproductive biology in shrews is rather well known including aspects such as induced ovulation (Bedford et al., 1997b), copulation behaviour (Dryden, 1969; Clendenon and Rissman, 1990), hormonal levels (Rissman and Crews, 1988; Veney and Rissman, 1998), multiple paternity (Searle, 1990; Stockley et al., 1993), sperm morphology and sperm distribution in the female genital tract (Bedford et al., 1994, 2004). In regard to sperm competition, our previous studies (Parapanov et al., 2007, 2008a, b) demonstrated that the higher relative testis size (testes/body percent) is associated with a shorter cycle length of spermatogenesis, increasing the rate of sperm production.

The aim of the present study was to investigate whether sperm numbers and sperm quality among four shrew species belonging to four genera are related to testis size, and behave in agreement with the sperm competition hypothesis. In particular, we investigated whether an increased relative testis size was associated with a higher number of epididymal spermatozoa, longer spermatozoa, higher sperm motility, and a higher concentration of plasma testosterone.

2. Materials and methods

2.1. Animals

Adult males of four species of shrews belonging to two subfamilies (Dubey et al., 2007), Soricinae (*Sorex araneus*, *Neomys fodiens*) and Crocidurinae (*Crocidura russula*, *Suncus murinus*) were used for comparative assessment of sperm production (Fig. 1).

From 2004 to 2006, we captured 16 adult male *S. araneus* chromosomal race Valais, now considered as *Sorex antinorii* (Brünner and Hausser, 1996), 14 *N. fodiens*, and 20 *C. russula*. The *Sorex* were captured in the regions of Trient and Grand St-Bernard in the Alps, *Neomys* and *Crocidura* in the regions of Lausanne and Champtauroz, Switzerland. The shrews were trapped after having reached sexual maturity in nature in the second calendar year of life, in spring or in early summer and were therefore of similar age and physiological state. All wild animals were kept in a dedicated building with a roof protecting them from direct rain, but with walls of wire mesh that exposed the animals to natural fluctuations of temperature and humidity according to the weather. The shrews were caged individually, on natural soil. They had *ad libitum* access to both water and food composed of minced meat with a vitamin supplement and mealworms. For *N. fodiens* we added also pieces of fish and dried *Gammarus*.

Nine adult male Asian musk shrews *S. murinus* (at least 2 months of age) were also used in this study. This subtropical species attains maturity rapidly after weaning (Dryden, 1969; Veney and Rissman, 1998). The animals were born and raised in our colony at the University of Lausanne. The colony founders were provided in 2005 by Prof. K. Tsuji and were descended from a colony at the University of Nagoya, Japan. The colony was maintained on a light cycle of 12 L:12D at a temperature of $25 \pm 1\,^{\circ}C$.

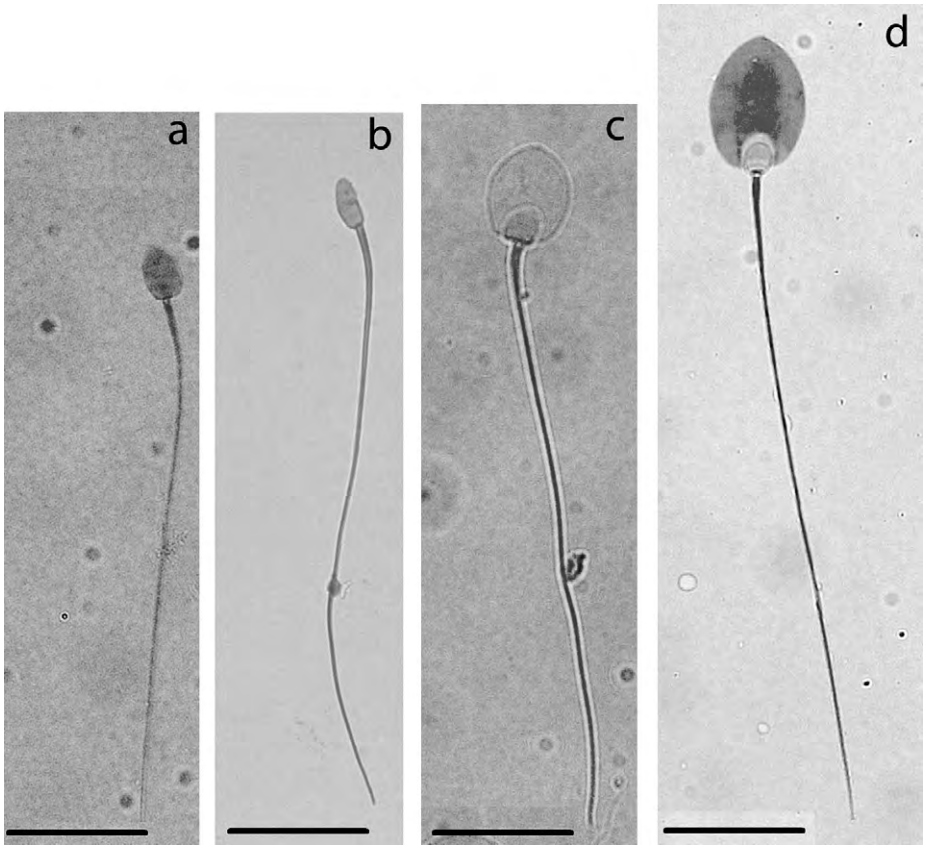


Fig. 1. Shrew spermatozoa released from cauda epididymis: *Sorex araneus* (a), *Neomys fodiens* (b), *Crocidura russula* (c), and *Suncus murinus* (d). Bars = 20 μm.

The shrews were caged individually, on natural soil and provided with food composed of minced meat with vitamin supplement, mealworms, dried *Gammarus*, fish pellets and water *ad libitum*.

2.2. Collection of epididymal spermatozoa and sperm count

All animals were killed by halothane overdose and weighed. Testes were removed and weighed. Both cauda epididymes were removed and placed each in a four-well (no. 176740, NUNCLON®, Nunc, Denmark), containing 1 ml of Dulbecco's Modified Eagle's Medium (DMEM-Sigma–Aldrich) with 3 mg/ml BSA pre-warmed at 37 °C. The cauda epididymis were gently ripped and minced until all the content of the tubules seemed to have been released. Aliquots for the motility analysis were removed immediately and then the dishes with the sperm suspensions were incubated at 37 °C under 5% CO_2 for up to 15 min.

To determine the sperm number, a 10 μl aliquot of the sample from each cauda epididymis was diluted in 10 μl of 4% formalin solution and spermatozoa were counted in improved Neubauer chamber (MARIENFELD, Germany). The total number of cauda epididymal spermatozoa for each animal was calculated.

2.3. Motility evaluation

Five males for each species were used for sperm motility analysis. The sperm aliquots were removed immediately from the dishes with the minced cauda epididymis and placed in a 20 μm depth analysis

chamber provided by "Leja Products" (no. SC20-01-FA, Netherlands), pre-warmed to 37 °C. Video sequence acquisition was performed by a digital camera "BASLER" 312 f/C (25 frames/s). The samples were video-recorded under dark field illumination for later analysis by computer-assisted sperm analysis (CASA), using the Sperm Class Analyzer® (Microptic S.L. Barcelona, Spain). This analysis consisted of determining the percentage of motile spermatozoa as well as the percent of progressively motile, non-progressive and static sperm. In addition, we determined sperm motility parameters, which are, curvilinear velocity (VCL), straight-line velocity (VSL), path average velocity (VAP) and linearity (LIN) in both soricine species. In the case of *S. murinus* and *C. russula* the computer-assisted sperm analysis could not be applied because it was not possible to adjust the software parameters to the large sperm head. Thus the sperm motility was assessed by subjective analysis of the video-recorded sequences.

2.4. Sperm morphometry

Sperm smears for morphometric measurements were made after sperm incubation. The slides were stained using Spermac Stain™ (FertiPro N.V., Beernem, Belgium) and then examined at a magnification of 630× on Axiophot microscope (Zeiss, Germany). Sperm morphometric data were obtained using AxioVision 4 AC software (Zeiss, Germany). Approximately 50 spermatozoa were analyzed per male for each species (5–8 males from each species). The morphometric parameters measured were: head length, head width, tail length and sperm total length (μm, mean ± S.E.).

2.5. Measurement of plasma testosterone concentrations

Blood samples were collected by cardiac puncture into heparinised tubes and centrifuged (1500 × *g* for 20 min). The plasma was decanted and stored at −20 °C until assay. Plasma concentrations in ng/ml of total testosterone were measured by enzyme-linked immunospecific assay according to manufacture procedure (IBL Testosterone ELISA kit, RE52151, Hamburg, Germany). We used 9–10 males for each species for testosterone determination.

2.6. Statistical analyses

All data are presented as means ± S.E. and all analyses were performed with R software (R Development Core Team, 2007). For comparison between soricine and crocidurine, we used mixed linear regression with subfamily as fixed effect and species as random effects. The significance level was considered to be 0.05, but *p*-values between 0.1 and 0.05 were regarded as tendencies because variation is high when dealing with living organisms and low sample size.

2.7. Ethical considerations

This project was executed under authorization of the State Commission for Animal Experiments of Canton de Vaud (1707.1). The number of samples of wild shrews was kept as low as possible because all species of shrews are protected animals in Switzerland.

3. Results

3.1. Body mass, testes mass and relative testis size

Measurements such as body mass, testes mass and relative testis size are given in Table 1 and Fig. 2. The soricine species *S. araneus* and *N. fodiens* have large testes (both testes) in relation to their body size, respectively 1.6 ± 0.23% and 1.5 ± 0.17%. *S. araneus* is characterized by the highest relative testis size. The crocidurine shrews *C. russula* and *S. murinus* have small testes in relation to their body size, respectively 0.3 ± 0.05% and 0.2 ± 0.04%. *S. murinus* had the smallest relative testis size. The difference in the relative testis size between our soricine and crocidurine shrews was highly significant ($p = 0.0001$). The within

Table 1
Characteristics of body mass, testes mass, relative testes size, total and relative sperm number in cauda epididymis and circulating testosterone level in four shrew species.

Species	Body mass (g)	Testes mass (g)	Testes/body (%)	Total sperm no. in cauda epididymes ($\times 10^6$)	Sperm no. per (g) body mass ($\times 10^6$)	Circulating testosterone (ng/ml)
S. araneus	10.9 ± 0.8 (n = 16)	0.17 ± 0.02 (n = 16)	1.6 ± 0.23 (n = 16)	13.2 ± 4.4 (n = 16)	1.2 ± 0.42 (n = 16)	7.9 ± 5.7 (n = 10)
N. fodiens	16.7 ± 1.6 (n = 14)	0.25 ± 0.03 (n = 14)	1.5 ± 0.17 (n = 14)	30.1 ± 14.2 (n = 14)	1.8 ± 0.89 (n = 14)	5.9 ± 4.0 (n = 10)
C. russula	13.4 ± 1.6 (n = 12)	0.04 ± 0.01 (n = 12)	0.3 ± 0.05 (n = 12)	1.7 ± 0.7 (n = 12)	0.13 ± 0.06 (n = 12)	1.1 ± 0.7 (n = 10)
S. murinus	66.1 ± 3.1 (n = 9)	0.15 ± 0.02 (n = 9)	0.2 ± 0.04 (n = 9)	3.3 ± 1.4 (n = 9)	0.05 ± 0.02 (n = 9)	1.9 ± 1.1 (n = 9)

Values are mean ± S.E., n = number of animals.

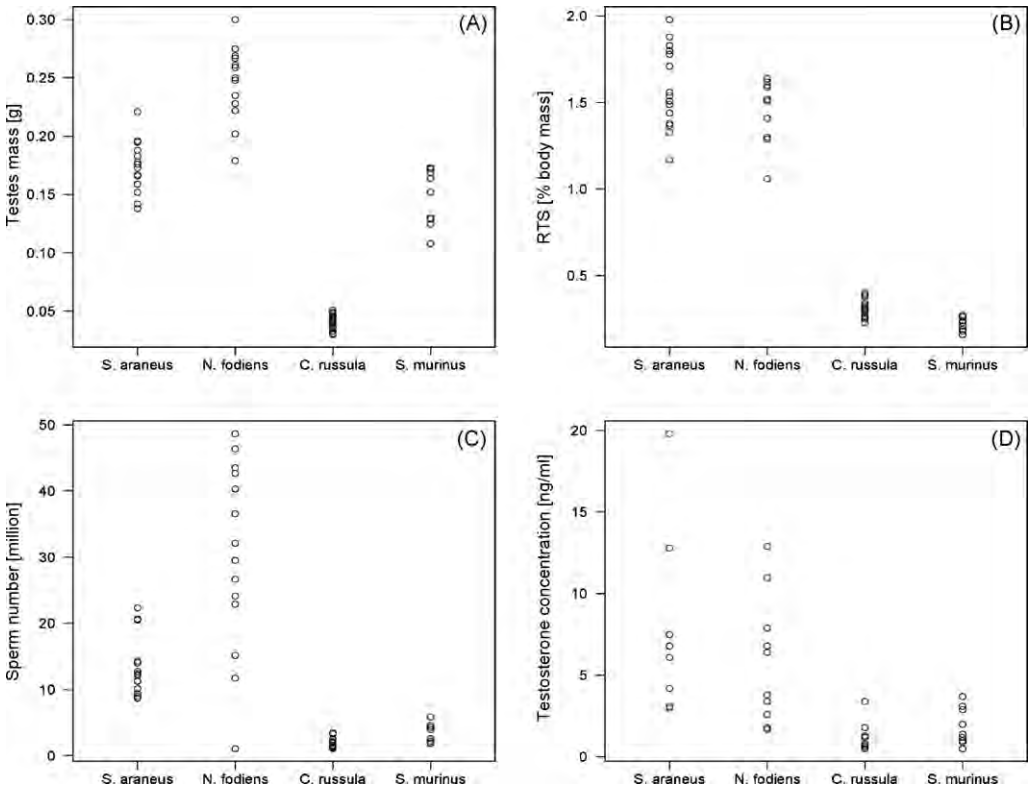


Fig. 2. Intra-specific variance of factors: testes mass (A), relative testes size (B), sperm number in the cauda epididymis (C) and testosterone concentrations (D).

species variation (Fig. 2.) is higher in species with high level of sperm competition (high RTS) than in species with low level of sperm competition (low RTS).

3.2. Total and relative number of epididymal spermatozoa

The total numbers of spermatozoa (Table 1) in the soricine shrews *S. araneus* ($13.2 \pm 4.4 \times 10^6$) and *N. fodiens* ($30.1 \pm 14.2 \times 10^6$) was significantly higher ($p = 0.036$) than in the crocidurine shrews *C. russula* ($1.7 \pm 0.7 \times 10^6$) and *S. murinus* ($3.4 \pm 1.4 \times 10^6$). The sperm numbers relative to body mass (Table 1) in both soricine shrews were significantly higher ($p = 0.012$) than in crocidurine shrews.

3.3. Sperm motility

The cauda epididymal spermatozoa of the four species displayed different patterns of motility (Table 2). The spermatozoa of both soricine species (*S. araneus*, *N. fodiens*) were characterized by high percentage of progressive motile spermatozoa ($82.3 \pm 10.6\%$ and $74.6 \pm 8.3\%$) and few non-progressive motile sperm ($4.2 \pm 2.6\%$ and $0.2 \pm 0.6\%$). In contrast, crocidurine species (*C. russula*, *S. murinus*) were characterized by low percentage of progressive motile sperm ($2.8 \pm 1.7\%$ and $17.6 \pm 7.0\%$) and high percentage of non-progressive motile sperm ($79.4 \pm 11.6\%$ and $39.6 \pm 4.5\%$). In addition, in both soricine species we measured sperm motility parameters. The spermatozoa of *S. araneus* were characterized by higher values for sperm motility parameters than those of *N. fodiens* (Table 2). In terms of sperm kinematics parameters, the spermatozoa of *C. russula* and *S. murinus* are characterized by a high degree

Table 2
Sperm motility parameters in four species of shrews.

	S. araneus ($n=5$)	*N. fodiens* ($n=5$)	*C. russula* ($n=5$)	*S. murinus* ($n=5$)
Motile sperm (%)	86.5 ± 8.6	74.8 ± 8.4	82.2 ± 12.5	57.2 ± 5.9
Sperm progressive motility (%)	82.3 ± 10.6	74.6 ± 8.3	2.8 ± 1.7	17.6 ± 7.0
Non-progressive sperm (%)	4.2 ± 2.6	0.2 ± 0.6	79.4 ± 11.6	39.6 ± 4.5
Static sperm (%)	13.5 ± 8.6	25.2 ± 8.4	17.8 ± 12.5	42.8 ± 5.9
Sperm parameters				
Curvilinear velocity (VCL) ($\mu m\,s^{-1}$)	148.2 ± 11.1	116.4 ± 4.0		
Straight-line velocity (VSL) ($\mu m\,s^{-1}$)	43.9 ± 9.0	28.7 ± 0.8		
Path average velocity (VAP) ($\mu m\,s^{-1}$)	59.8 ± 7.7	47.2 ± 2.0		
Linearity (LIN) (VSL/VCL × 100)	29.4 ± 3.8	24.6 ± 0.7		

Values are mean ± S.E., n = number of animals.

Table 3
Sperm morphometric parameters in four species of shrews.

	S. araneus ($n=5$)	*N. fodiens* ($n=6$)	*C. russula* ($n=8$)	*S. murinus* ($n=5$)
Head length	7.5 ± 0.4	7.2 ± 0.3	14.5 ± 0.6	18.1 ± 1.0
Tail length	76.4 ± 0.9	86.7 ± 0.9	88.3 ± 1.4	95.3 ± 1.3
Total sperm length	83.9 ± 1.1	93.9 ± 0.9	102.7 ± 1.5	113.3 ± 1.8
Head width	5.1 ± 0.4	2.9 ± 0.3	12.3 ± 0.4	13.7 ± 0.9

Values are in μm mean ± S.E., n = number of animals.

of undulatory displacement of flagellum while the sperm heads were stacked on the bottom of the chamber and did not progress.

3.4. Sperm morphometry

Table 3 shows the values for spermatozoa morphometric parameters. A comparison of the sperm parameters of the Soricinae and Crocidurinae showed a clear difference. The values for the head length ($p=0.002$), head width ($p=0.0005$) and total sperm length ($p=0.021$), were significantly higher in *C. russula* and *S. murinus* than those in *S. araneus* and *N. fodiens*.

3.5. Plasma concentrations of testosterone

Plasma testosterone concentrations were measured in order to determine the relationship between sperm production and testosterone concentrations. Plasma testosterone concentration (Table 1) were significantly higher in *S. araneus* 7.9 ng ml^{-1} and *N. fodiens* 5.9 ng ml^{-1} than in *C. russula* 1.1 ng ml^{-1} and *S. murinus* 1.9 ng ml^{-1} ($p=0.0021$).

4. Discussion

It is well established that female promiscuity can lead to sperm competition which is defined as postcopulatory competition between the spermatozoa of two or more males to fertilise female ova (Parker, 1970; Møller and Birkhead, 1989). The most obvious evidence of sperm competition is the evolution of large testes and a high number of produced sperm. In shrews, our comparative studies (Parapanov et al., 2007, 2008a) suggest that the selective pressure of sperm competition has not only favored the evolution of great testis size in relation to body size but moreover a shorter cycle length of spermatogenesis in order to increase sperm production.

The results of the present study confirm, as expected, that the relative testis size is positively associated with the number of sperm stored in the cauda epididymis (Table 1). The difference in the number of spermatozoa between our studied species of shrews is unequivocal and reflects their mating system. Concerning the testes mass and sperm number, our results are in agreement with the findings in other shrew species such as in *Crocudura suaveolens* (Bedford et al., 1997c) called by error *C. russula monacha*

(Dubey et al., 2007) and *Cryptotis parva* (Bedford et al., 1997a). In rodents the relative testis size is also related to the number of sperm stored in the male tract (Pierce et al., 1990; Peirce and Breed, 2001; Gomendio et al., 2006).

In the context of sperm properties, the sperm motility, as characterized by sperm velocity and the proportion of mobile spermatozoa, might also influence the fertilization outcome (Birkhead et al., 1999; Gage et al., 2004). When the sperm from rival males compete, sperm velocity may be crucial to determine which spermatozoa arrive first and have an increased chance of fertilizing the ova. Moreover, a high percentage of progressive motile spermatozoa might also contribute to create an advantageous outcome. As a conclusion, sperm competition should enhance sperm motility parameters in the species of shrews characterized by high level of sperm competition. As a matter of fact, in *S. araneus* and *N. fodiens* the cauda epididymal spermatozoa were characterized by a high progressive motility. It is likely that in this stage of post-testicular development the spermatozoa had acquired the capacity for progressive motility. In both crocidurine species *S. murinus* and *C. russula* our findings about the sperm motility of the cauda epididymal spermatozoa were similar to those described previously by Bedford et al. (1994). The majority of the sperm was motile but did not progress. This motility pattern exhibited by the cauda epididymal spermatozoa might indicate that the spermatozoa of crocidurine shrews (*S. murinus* and *C. russula*) acquire their capacity for progressive motility later in their post-testicular development (Bedford et al., 1994).

A possible artifact related to the *in vitro* conditions used to determine the sperm mobility cannot be totally excluded. We therefore also tested two other media (Tyrode's salt solution and M199) but without different results (not shown). It should be noted here that the ejaculated spermatozoa collected after copulation from female genital tract (vagina and uterus) in both *C. russula* and *S. murinus* showed high progressive motility (unpublished data). The comparison between the two subfamilies allows us to postulate that sperm competition has influenced post-testicular maturation in order to enhance success in a competitive mating system.

A direct comparison between sperm motility parameters in crocidurine and soricine species is problematic because of very different sperm characteristics. The head shape of spermatozoa may also be important in influencing sperm swimming velocity; a very large head may strongly decrease sperm motility.

Many studies have attempted to understand if the sperm competition influences the sperm size and sperm design (Malo et al., 2006; Gomendio et al., 2007; Immler and Birkhead, 2007). In both primates and rodents, the males from polyandrous species have longer spermatozoa than males from monoandrous species (Gomendio and Roldan, 1991). In birds, postcopulatory sperm competition favors longer and more elaborate sperm (Briskie and Montgomerie, 1992; Briskie et al., 1997). In addition, Calhim et al. (2007) showed in 18 species of passerine birds, that postcopulatory sexual selection favors the longer sperm.

By contrast, in primates, Harcourt (1991) found no relationship between mating system and sperm length. Gage and Freckleton (2003) also showed across 83 mammalian species no evidence for associations between sperm competition and sperm length. In regard to our shrew species, we evidenced that the longer spermatozoa were associated with the small relative testis size, an indicator suggesting rather a monogamous mating system.

According to the head shape, the shrew's spermatozoa can be separated generally into two groups (Bedford et al., 1994). The first one is characterized by modest acrosome size such as in *S. araneus* described by Plöen et al. (1979). We found a similar acrosomal shape in other soricine species such as *Sorex coronatus* and *Sorex minutus* (unpublished data). Based on the sperm head shape, spermatozoa of *N. fodiens* might be also added to this group. The second group is characterized by unusually large fan-like acrosome and was described in crocidurine, *S. murinus* (Cooper and Bedford, 1976) and *Crocidura suaveolens* (Bedford et al., 1997c). The spermatozoa of *C. russula* are characterized by similar head shape as other studied crocidurine species and can be added the second group. This variation of sperm morphology in shrews is difficult to understand and raises the as yet unanswered question of the role of the acrosome during the sperm penetration of the female ova (Bedford, 2004; Bedford et al., 2004). It is possible that the different head design could be the result of another evolutionary requirement.

The consequences of sperm competition extend far beyond the evolution of larger relative testis size and selection for the production of large numbers of motile spermatozoa. Dixson and Anderson

(2004) have reported a relationship between circulating testosterone levels, sexual behaviour and mate competition. Other authors have reported that, in birds and in Old World monkeys, the testosterone levels are associated with the mating systems; being lower in monogamous than in polygamous species (Wingfield et al., 1990; Whitten, 2000). Our study also demonstrated that inter-specific differences in terms of circulating testosterone concentrations are closely associated to relative testis size and might be also interpreted by the sperm competition hypothesis.

In this study, we also looked at intra-specific variation in testes mass, relative testes mass, epididymal sperm number and circulating testosterone presented in Fig. 2. The values varied more within the two species with high level of sperm competition (high RTS) than in the two species with low level of sperm competition (low RTS). While the variation could be due to different natural parasite burdens in these wild-caught shrews, the wild-caught *C. russula* did not show similar variance. On the other hand the higher variance within the Soricinae may be due to the competitive conditions which lead the males to adjust their reproductive output according to requirements, in order to maintain or increase their competitiveness. In the non-competitive Crocidurinae, the males do not experience this selective pressure and the reproductive parameters are more similar.

5. Conclusion

Finally, if the sperm competition influences the sperm production, it is logical to assume that it acts on all parts of the male reproductive system, including associated physiological mechanisms (accessory glands, endocrinology) and sexual behaviour. Here, we studied only some traits of sperm quality. However, to better understand sperm competition we also have to take into account the role of female genital tract, the place where the competition between spermatozoa of different males occurs. In conclusion, our study provides some new information on sperm production and sperm properties in shrews which provides further insight into how sperm competition influences mammalian species.

References

Aslam, H., Schneiders, A., Perret, M., Weinbauer, G.F., Hodges, J.K., 2002. Quantitative assessment of testicular germ cell production and kinematic and morphometric parameters of ejaculated spermatozoa in the grey mouse lemur, *Microcebus murinus*. Reproduction 123, 323–332.

Bedford, J.M., 2004. Enigmas of mammalian gamete form and function. Biol. Rev. Camb. Philos. Soc. 79, 429–460.

Bedford, J.M., Cooper, G.W., Phillips, D.M., Dryden, G.L., 1994. Distinctive features of the gametes and reproductive tracts of the Asian musk shrew, *Suncus murinus*. Biol. Reprod. 50, 820–834.

Bedford, J.M., Mock, O.B., Goodman, S.M., 2004. Novelties of conception in insectivorous mammals (Lipotyphla), particularly shrews. Biol. Rev. Camb. Philos. Soc. 79, 891–909.

Bedford, J.M., Mock, O.B., Phillips, D.M., 1997a. Unusual ampullary sperm crypts, and behavior and role of the cumulus oophorus, in the oviduct of the least shrew, *Cryptotis parva*. Biol. Reprod. 56, 1255–1267.

Bedford, J.M., Mori, T., Oda, S., 1997b. Ovulation induction and gamete transport in the female tract of the musk shrew, *Suncus murinus*. J. Reprod. Fertil. 110, 115–125.

Bedford, J.M., Phillips, D.M., Mover Lev, H., 1997c. Novel sperm crypts and behavior of gametes in the fallopian tube of the white-toothed shrew, *Crocidura russula monacha*. J. Exp. Zool. 277, 262–273.

Birkhead, T.R., Martinéz, J.G., Burke, T., Froman, D.P., 1999. Sperm mobility determines the outcome of sperm competition in the domestic fowl. Proc. Roy. Soc. Lond. B: Biol. Sci. 266, 1759–1764.

Briskie, J.V., Montgomerie, R., 1992. Sperm size and sperm competition in birds. Proc. Roy. Soc. Lond. B: Biol. Sci. 247, 89–95.

Briskie, J.V., Montgomerie, R., Birkhead, T.R., 1997. The evolution of sperm size in birds. Evolution 51, 937–945.

Brünner, H., Hausser, J., 1996. Genetic and karyotypic structure of a hybrid zone between the chromosomal races Cordon and Valais in the common shrew, *Sorex araneus*. Hereditas 125, 147–158.

Calhim, S., Immler, S. Birkhead, T.R. 2007. Postcopulatory sexual selection is associated with reduced variation in sperm morphology. PLoS ONE. 2, e413. doi:10.1371/journal.pone0000413.

Clendenon, A.L., Rissman, E.F., 1990. Prolonged copulatory behaviour facilitates pregnancy in the musk shrew. Physiol. Behav. 47, 831–835.

Cooper, G.W., Bedford, J.M., 1976. Asymmetry of spermiation and sperm surface charge patterns over the giant acrosome in the musk shrew *Suncus murinus*. J. Cell Biol. 69, 415–428.

Dixson, A.F., Anderson, M.J., 2004. Sexual behaviour, reproductive physiology and sperm competition in male mammals. Physiol. Behav. 83, 361–371.

Dryden, G.L., 1969. Reproduction in *Suncus murinus*. J. Reprod. Fertil. Suppl. 6, 377–396.

Dubey, S., Salamin, N., Ohdachi, S.D., Barrière, P., Vogel, P., 2007. Molecular phylogenetics of shrews (Mammalia: Soricidae) reveal timing of transcontinental colonization. Mol. Phylogenet. Evol. 44, 126–137.

Gage, M.J.G., Freckleton, R.P., 2003. Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. Proc. Roy. Soc. Lond. B 270, 625–632.

Gage, M.J.G., Macfarlane, C.P., Yeates, S., Ward, R.G., Searl, J.B., Parker, G.A., 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. Curr. Biol. 14, 44–47.
Gomendio, M., Malo, A.F., Garde, J., Roldan, E.R., 2007. Sperm traits and male fertility in natural populations. Reproduction 134, 19–29.
Gomendio, M., Martin-Coello, J., Crespo, C., Magaña, C., Roldan, E.R., 2006. Sperm competition enhances functional capacity of mammalian spermatozoa. Proc. Natl. Acad. Sci. U.S.A. 103, 15113–15117.
Gomendio, M., Roldan, E.R., 1991. Sperm competition influences sperm size in mammals. Proc. Biol. Sci. 243, 181–185.
Gomendio, M., Roldan, E.R., 1993. Mechanisms of sperm competition: linking of physiology and behavioural ecology. Trends Ecol. Evol., 95–100.
Harcourt, A.H., 1991. Sperm competition and the evolution of nonfertilising sperm in mammals. Evolution 45, 314–328.
Harcourt, A.H., Harvey, P.H., Larson, S.G., Short, R.V., 1981. Testis weight, body weight and breeding system in primates. Nature 293, 55–57.
Harvey, P.H., Harcourt, A.H., 1984. Sperm competition, testes size and breeding systems in primates. In: Smith, R.L. (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York, pp. 589–600.
Hunter, F.M., Birkhead, T.R., 2002. Sperm viability and sperm competition in insects. Curr. Biol. 12, 121–123.
Immler, S., Birkhead, T.R., 2007. Sperm competition and sperm midpiece size: no consistent pattern in passerine birds. Proc. Biol. Sci. 274, 561–568.
Kenagy, G.J., Trombulak, S.C., 1986. Size and function of mammalian testes in relation to body size. J. Mammal. 67, 1–22.
Malo, A.F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A.J., Roldan, E.R., 2006. Sperm design and sperm function. Biol. Lett. 2, 246–249.
Møller, A.P., Birkhead, T.R., 1989. Copulation behaviour in mammals: evidence that sperm competition is widespread. Biol. J. Linn. Soc. 38, 119–131.
Parapanov, R., Nussle, S., Hausser, J., Vogel, P., 2008a. Relationships of basal metabilic rate, relative testis size and cycle length of spermatogenesis in shrews (Mammalia, Soricidae). Reprod. Fertil. Dev. 20, 431–439.
Parapanov, R., Nussle, S., Hausser, J., Vogel, P., 2008b. Histological description of seminiferous epithelium and cycle length of spermatogenesis in the water shrew *Neomys fodiens* (Mammalia: Soricidae). Anim. Reprod. Sci. 107, 148–160.
Parapanov, R., Nussle, S., Vogel, P., 2007. Cycle length of spermatogenesis in shrews (Mammalia: Soricidae) with high and low metabolic rates and different mating systems. Biol. Reprod. 76, 833–840.
Parker, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45, 525–567.
Parker, G.A., 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. J. Theor. Biol. 96, 281–294.
Peirce, E.J., Breed, W.G., 2001. A comparative study of sperm production in two species of Australian arid zone rodents (*Pseudomys australis*, *Notomys alexis*) with marked differences in testis size. Reproduction 121, 239–247.
Pierce, J.D., Ferguson, B., Salo, A.L., Sawrey, D.K., Shapiro, L.E., Taylor, S.A., Dewsbury, D.A., 1990. Patterns of sperm allocation across successive ejaculates in four species of voles (Microtus). J. Reprod. Fertil. 88, 141–149.
Plöen, L., Ekwall, H., Afzelius, B.A., 1979. Spermiogenesis and the spermatozoa of the European common shrew (*Sorex araneus* L.). J. Ultrastruct. Res. 68, 149–159.
Ramm, S.A., Parker, G.A., Stockley, P., 2005. Sperm competition and the evolution of the male reproductive anatomy in rodents. Proc. Roy. Soc. Biol. Sci. 272, 949–955.
Rissman, E.F., Crews, D., 1988. Hormonal correlates of sexual behavior in the female musk shrew: the role of estradiol. Physiol. Behav. 44, 1–7.
Searle, J.B., 1990. Evidence for multiple paternity in the common shrew (*Sorex araneus*). J. Mammal. 71, 139–144.
Stockley, P., Gage, M.J.G., Parker, G.A., Moller, A.P., 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. Am. Nat. 149, 933–954.
Stockley, P., Searle, J.B., Macdonald, D.W., Jones, C.S., 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. Proc. Roy. Soc. Lond. B 254, 173–179.
Veney, S.L., Rissman, E.F., 1998. Co-localization of estrogen receptor and aromatase enzyme immunoreactivities in adult musk shrew brain. Horm. Behav. 33, 151–162.
Whitten, P.L., 2000. Evolutionary endocrinology of the cercopithecoides. In: Whitehead, P.F., Jolly, C.J. (Eds.), Old World Monkeys. Cambridge University Press, Cambridge, UK, pp. 269–297.
Wingfield, J.C., Henger, R.E., Duffy, A.M., Ball, G.F., 1990. The "challenger hypothesis": theoretical implications for patterns of testosterone selection, mating systems and breeding strategies. Am. Nat. 136, 829–846.