The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2006, 15: 309-313
ISSN 0962-7286

The effect of cover on food consumption and growth in two freshwater fish species used in experimental studies

RJ Wootton*, N Handisyde and C Rowe

Institute of Biological Sciences, University of Wales Aberystwyth, Aberystwyth SY23 3DA, Wales, UK
* Contact for correspondence and requests for reprints: rjw@aber.ac.uk

Abstract

*To define suitable holding conditions for fish used in experimental studies on food consumption and growth, the effect of cover on growth at two ration levels was quantified for immature individuals of two common, freshwater species: the threespine stickleback (*Gasterosteus aculeatus*: Gasterosteidae) and the European minnow (*Phoxinus phoxinus*: Cyprinidae). In the European minnow, the presence of cover significantly increased growth rates and also the rate of food consumption at* ad libitum *rations; however, cover had no significant effect on the growth of the threespine stickleback. In both species, fish fed* ad libitum *rations had higher growth rates than fish fed 4% of body weight per day. This study illustrates the need for species specific holding conditions and illustrates an approach to welfare using measurements of traits closely related to fitness.*

Keywords: *animal welfare, appetite, condition, European minnow, specific growth rate, threespine stickleback*

Introduction

Although teleost fish are used as a resource in commercial and recreational fisheries, aquaculture, the aquarist trade and scientific research, their welfare requirements have received far less attention than those of endothermic vertebrates. However, interest in the welfare of teleost fish is increasing; for example, in 2002 the Fisheries Society of the British Isles issued a document on fish welfare (FSBI 2002). There is an immediate need to define the best conditions for holding fish used in scientific research to ensure that the results are not invalidated because the welfare of the experimental animals has been compromised. This need has become more acute because of the popularity of teleost models, such as the zebra fish (*Danio rerio*) and the medaka (*Oryzias latipes*), in genomic research.

A functional approach to the problem is to determine the effect of holding conditions on components of fitness, including the growth, survival and fecundity of the individuals. The assumption is that poor welfare of fish will compromise their performance (FSBI 2002). As part of a study on the effects of changing availability of food on the growth and fecundity of fish (eg Russell & Wootton 1992; Ali & Wootton 1999, 2001), the effect of cover on the rate of food consumption and growth rate was studied experimentally in two freshwater species indigenous to the British Isles, the threespine stickleback (*Gasterosteus aculeatus*: Gasterosteidae) and the European minnow (*Phoxinus phoxinus*: Cyprinidae). Both species are widely distributed and common, and both are extensively used in experimental studies on fish (Winfield & Nelson 1991; Bell & Foster 1994). In particular, the threespine stickleback has become a model vertebrate species for the analysis of genetic effects on skeletal structure (Peichel *et al* 2001), sex determination (Peichel *et al* 2004) and growth rates (Wright *et al* 2004).

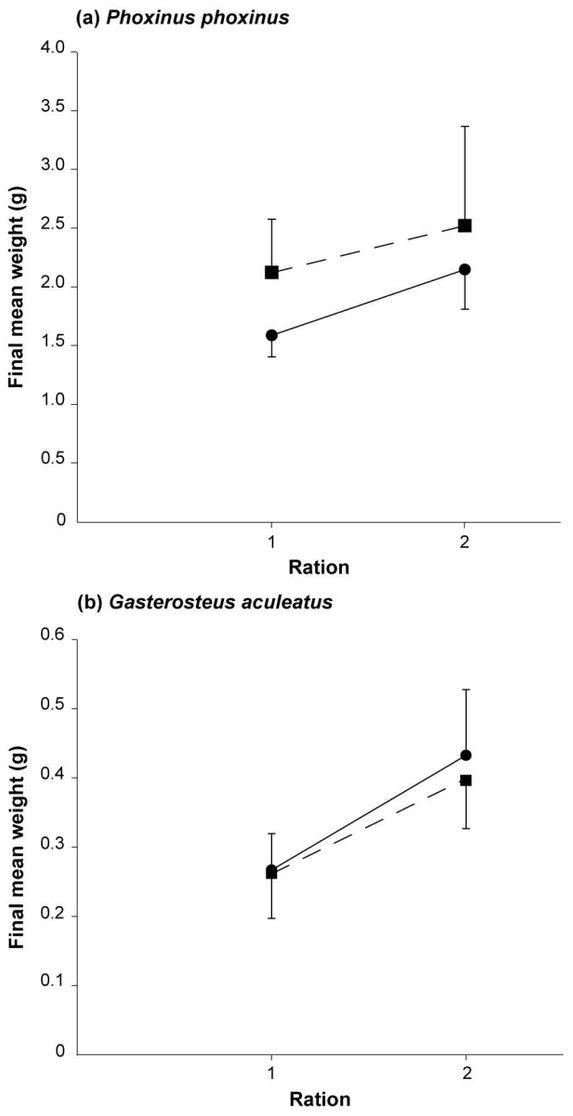
The aim of this study was to define suitable holding conditions for individually-housed fish in experimental studies in which the quantity of food consumed and growth were the experimental variables of interest.

Materials and methods

Immature threespine sticklebacks and European minnows were collected from Llyn Frongoch in mid-Wales and then held in single-species, communal tanks at a temperature of 14–16°C with a natural photoperiod in the aquarium of the Institute of Biological Sciences under UK Home Office Regulations. The fish were fed on a combination of live enchytraeid worms and commercial fish flakes until used in the experiment.

Thirty-six fish of each species were individually transferred to 20-litre tanks located in two constant environment rooms, which allowed the temperature to be controlled at 15 ± 0.5°C. Lighting was provided by fluorescent tubes above the tanks, giving a light intensity of 300 lux at water level, with a photoperiod of 10 h:14 h, light:dark. These conditions ensured that the fish remained sexually immature. The individual tanks were housed in four re-circulating systems, with each system containing nine tanks. In each system, the re-circulated water was pumped through a biological filter and was aerated to maintain 100% oxygen saturation. Although the fish were held individually, they could see other fish through the transparent sides of the tanks.

Figure 1



Effect of cover and ration on unadjusted mean final weight (g) of **(a)** the European minnow, *Phoxinus phoxinus*, and **(b)** the threespine stickleback, *Gasterosteus aculeatus*. Ration 1 = fed 4% of initial fresh body weight per day; Ration 2 = fed *ad libitum*; broken line = cover present; unbroken line = cover absent. Vertical line gives + or – 95% confidence interval.

For one week, all fish were fed *ad libitum* with live enchytraeid worms. Then, the fish were starved for 24 h to empty the alimentary canal. Fish were sedated in a 15 ppm solution of MS222 (tricaine methane sulphonate) (Ross & Ross 1999), carefully blotted to remove superficial water and then weighed to the nearest 0.1 mg. Vernier calipers were used to measure standard length (snout to end of caudal peduncle) to the nearest 0.1 mm by measuring the distance between pin pricks in an acetate sheet, which marked the position of the snout and caudal peduncle. After measurement, the fish were returned to their individual tanks.

Eighteen tanks, selected at random, contained half of a small terracotta flower pot. The pot provided cover for the fish and was sufficiently large for a fish to retreat entirely within it; 18 tanks did not contain a flower pot. The fish were then assigned, at random, to two ration levels of live enchytraeid worms: the *ad libitum* group had daily access to excess food, whereas the restricted group were fed 4% of their initial fresh body weight per day. A ration of 4% was sufficient to allow some growth under the prevailing temperature and photoperiod regimes, but was always less than a satiation ration (Allen & Wootton 1982; Cui & Wootton 1988). A weighed quantity of worms was added to each tank and any uneaten worms were recovered the following day and reweighed; therefore, the weight consumed by each individual fish was calculated by the difference. The experiment ran for 28 days, after which the fish were starved for 24 h, weighed and measured. The fish were then returned to single-species, communal tanks in the main aquarium. On return to their communal tanks, the fish fed normally and showed no adverse behavioural responses.

Daily specific growth rate (Gw), that is the instantaneous rate of increase per unit weight (ie dW/Wdt) was calculated as $100(ln\mathrm{WfWi}^{-1})t^{-1}$, where Wf and Wi are final and initial weights and t is the duration of the experiment in days (Wootton 1998). Fish condition was calculated as weight at length, obtained from the appropriate regression of weight on length (Wootton 1998).

Data were analysed by two-way ANOVA and ANCOVA with shelter and ration as factors, and initial length or weight as the covariate. Weekly food consumption was analysed with repeated-measures ANCOVA (Quinn & Keough 2002), with ration and cover as the between-subjects factors, week as the within-subjects factor and initial weight as the covariate. To stabilise variances, weights and lengths were log-transformed before analysis. The experimental design was balanced with nine replicates per treatment combination, but one stickleback in the *ad libitum*–no cover treatment died. Species was not used as a factor in the analyses because limitations of space precluded using the two species simultaneously and the two differed by an order of magnitude in initial size.

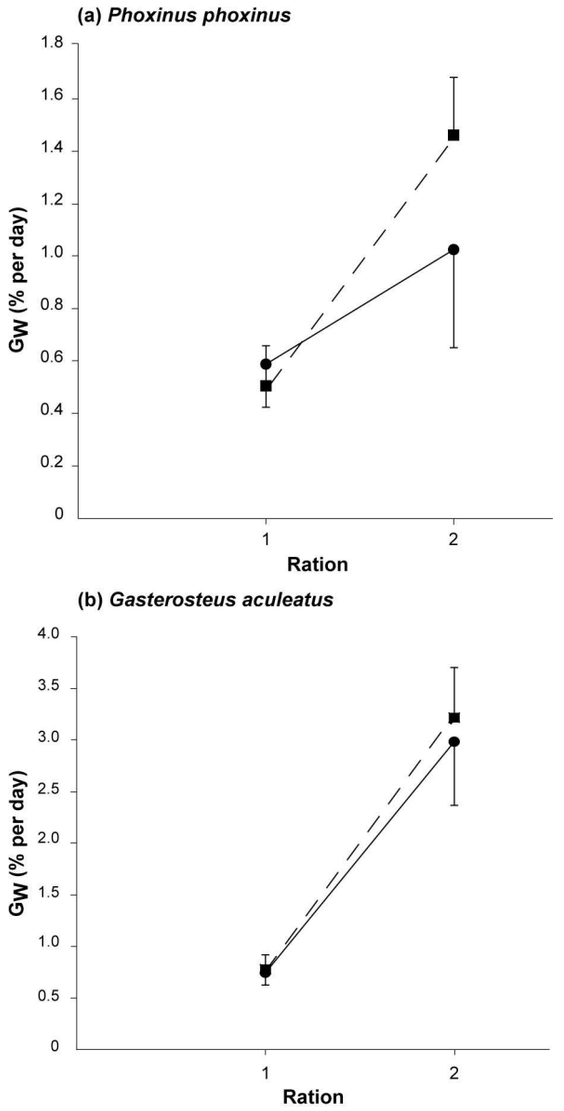
Results

European minnow

There were no significant differences in mean weight or length between treatments groups at the start of the experiment (initial mean weight ± SE = 1.717 ± 0.108 g; initial mean length = 57.1 ± 1.3 mm). However, at the end of the experiment, ANCOVA, with initial weight as the covariate, showed that both ration ($F_{1,31} = 53.06$, $P < 10^{-6}$) and cover ($F_{1,31} = 7.966$, $P = 0.008$) had a significant effect on final weight; final weight was significantly higher in fish fed the *ad libitum* ration and with cover (Figure 1a). There was no significant interaction between ration and cover ($F_{1,31} = 2.887$, $P = 0.10$). The mean Gw was significantly higher with cover ($F_{1,31} = 6.634$, $P = 0.01$) and *ad libitum* ($F_{1,31} = 44.72$, $P < 10^{-6}$) rations. The interaction between

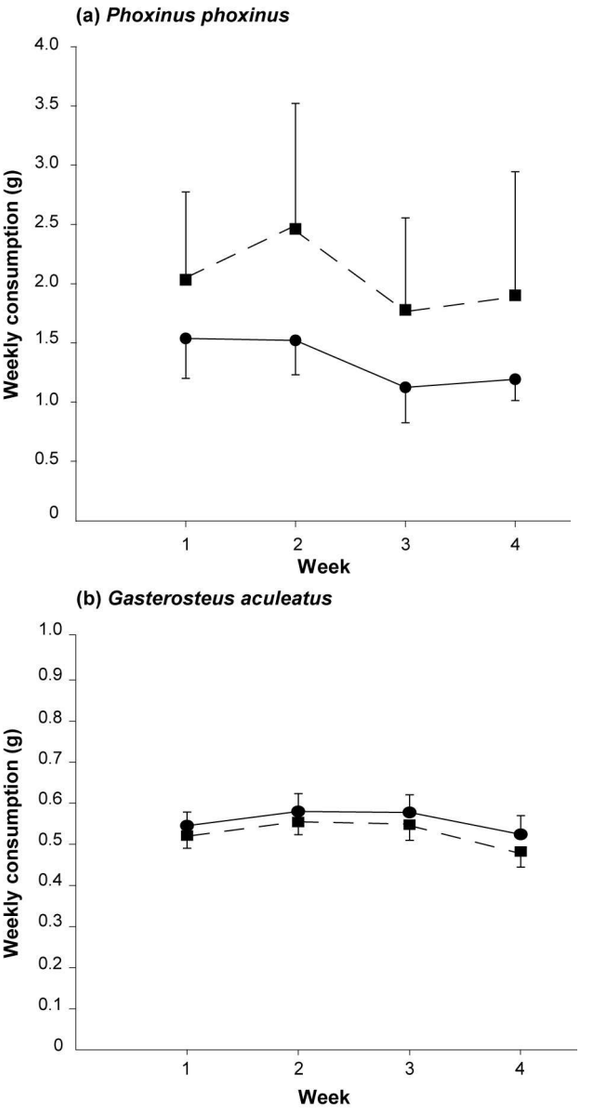
https://doi.org/10.1017/S0962728600030517 Published online by Cambridge University Press

Figure 2



Effect of cover and ration on unadjusted mean daily specific growth rate (Gw as % per day) of **(a)** the European minnow, *P. phoxinus*; and **(b)** the threespine stickleback, *G. aculeatus*. Symbols as in Figure 1.

Figure 3



Effect of cover on unadjusted mean weekly food consumption of **(a)** the European minnow, *P. phoxinus*; and **(b)** the threespine stickleback, *G. aculeatus*. Symbols as in Figure 1.

ration and cover approached significance ($F_{1,31}$ = 3.469, P = 0.07) (Figure 2a). Ration ($F_{1,31}$ = 41.70, $P < 10^{-6}$) and cover ($F_{1,31}$ = 7.234, P = 0.01) had significant effects on final fish condition; the presence of cover and *ad libitum* feeding both increased fish weight at a given length.

The European minnows fed the 4% ration ate all the food provided; therefore, an analysis of the effect of cover on food consumption was restricted to the *ad libitum* ration treatment. Cover ($F_{1,15}$ = 11.96, P = 0.004) and week ($F_{3,48}$ = 7.712, P = 0.0003) had significant effects on mean daily consumption. Consumption was higher by fish with cover and showed inter-week variations. There was no interaction between cover and weeks (Figure 3a).

Threespine stickleback

There were no significant differences in mean weight or length between treatment groups at the start of the experiment (initial mean weight + SE = 0.202 ± 0.034 g; initial mean length = 30.7 ± 5.2 mm). At the end of the experiment, fish fed the *ad libitum* ration had a significantly higher weight ($F_{1,30}$ = 142.5, $P < 10^{-6}$), but the effect of cover was not significant ($F_{1,30}$ = 0.05, P = 0.81), and the interaction between ration and cover was not significant ($F_{1,30}$ = 0.043, P = 0.84) (Figure 1b). Equally, only ration had a significant effect on Gw ($F_{1,30}$ = 18.49, $P < 10^{-6}$) (Figure 2b), and weight at a given length ($F_{1,30}$ = 15.77, P = 0.0004).

There was no significant effect of cover on mean daily food

https://doi.org/10.1017/S0962728600030517 Published online by Cambridge University Press

consumption ($F_{1,15} = 1.551$, $P = 0.23$), but there was significant week effect ($F_{3,45} = 18.48$, $P < 10^{-6}$) (Figure 3b).

Discussion

The aim of this study was to determine appropriate holding conditions for fish species that are to be used in experimental studies on the effect of variation in food supply on growth. The criteria used were variables closely related to Darwinian fitness, namely growth, condition and voluntary food consumption. It assumes that poor welfare conditions will be reflected in adverse effects on traits closely related to fitness. This approach to fish welfare, using accurately quantifiable criteria, avoids the problem of assessing the subjective state of individual fish (FSBI 2002; Rose 2002; Sneddon *et al* 2003; Braithwaite & Huntingford 2004; Sneddon 2004).

Both species used in this study, the threespine stickleback (*Gasterosteus aculeatus*) and the European minnow (*Phoxinus phoxinus*), fed and grew in the presence or absence of cover at both ration levels, but there were differences between the species. The European minnows ate more and grew faster in the presence of cover than in the absence of cover. Even when fed a fixed ration of 4% body weight per day, the minnows with cover grew faster than those without. No effect of cover was detected for the threespine stickleback.

The difference between the species in the effect of cover may relate to behavioural differences between the two. Although they are frequently found living in the same freshwater habitats, the minnow typically has a more pronounced tendency to form shoals. In Llyn Frongoch, the small reservoir from which the fish were collected, large shoals of minnows can be seen swimming in the shallows during the summer. Similar shoals of sticklebacks are rarely if ever seen (RJ Wootton personal observation). In the experiments on growth and feeding, the fish were housed individually so food consumption could be measured accurately and related to growth. For a minnow, an individual may be more prone to use cover if it is not part of a shoal. Threespine sticklebacks are renowned for their boldness and willingness to move into open areas (Wootton 1976; Huntingford *et al* 1994), and even when isolated, may be less prone to seek shelter and therefore benefit less from its presence. The lower growth rate of minnows in the absence of cover may reflect higher energy expenditure on locomotion, but no direct measurements of activity levels could be made.

Most other studies of the effects of cover on growth in fish have focused on ecological questions rather than welfare. Ecological studies usually explore the effects of cover in the presence of predators (eg Skov & Koed 2004) or conspecifics (eg Mikheev *et al* 1994). However, one of the few studies that examined the effect of cover *per se* in fish also found inter-specific differences (Fischer 2000a). The study compared the effect of differences in the nature of the substratum on the respiration rates of two benthic, freshwater species: the burbot (*Lota lota*) and the stone loach (*Barbatula barbatula*) (Fischer 2000a). During the day, burbot with a substratum of pebbles had higher rates of respiration than fish with a cobble substrate; the cobble substratum provided more cover than the pebble. In contrast, the respiration rate of the stone loach did not differ significantly with the type of substratum. Fischer (2000b) also demonstrated that burbot, fed *ad libitum*, had a significantly lower growth rate when on a pebble substratum than on a cobble substratum.

Animal welfare implications

These studies show that in fish components of fitness, such as growth, can be sensitive to details of the holding conditions, but there can be significant inter-specific differences. Given the restricted information available on the welfare requirements of fish used in experimental research, research programmes should consider incorporating preliminary studies on the optimal holding conditions for the species being used, in the context of the experiments that are to be implemented in the programme. In this way, a case history of best-practice for experimental studies on teleost fish can develop, while avoiding unresolvable discussions on the subjective experiences of fish.

Acknowledgements

C Rowe was supported by a University of Wales Aberystwyth student vacation grant. We are grateful to G Owen for help with the fish husbandry.

References

Ali M and Wootton RJ 1999 Coping with resource variability: effect of constant and variable intervals between feeding on reproductive performance at first spawning of female threespined sticklebacks, *Gasterosteus aculeatus* L. *Journal of Fish Biology 55*: 211-220

Ali M and Wootton RJ 2001 Capacity for growth compensation in juvenile three-spined sticklebacks experiencing cycles of food deprivation. *Journal of Fish Biology 58*: 1531-1544

Allen JRM and Wootton RJ 1982 The effect of ration and temperature on the growth of the threespine stickleback, *Gasterosteus aculeatus. Journal of Fish Biology 20*: 401-422

Bell MA and Foster SA (eds) 1994 *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press: Oxford, UK

Braithwaite VA and Huntingford FA 2004 Fish and welfare: do fish have the capacity for pain perception and suffering? *Animal Welfare 13, Suppl*: S87-S92

Cui Y and Wootton RJ 1988 Bioenergetics of growth of a cyprinid, *Phoxinus phoxinus* (L): the effect of ration and temperature of growth rate and efficiency. *Journal of Fish Biology 33*: 763-773

Fischer P 2000a An experimental test of metabolic and behavioural responses of benthic fish species to different types of substrate. *Canadian Journal of Fisheries and Aquatic Sciences 57*: 2336-2344

Fischer P 2000b Competitive interactions for space between the two benthic fish species, burbot (*Lota lota* L) and stone loach (*Barbatula barbatula* L). *Environmental Biology of Fishes 58*: 439-446

FSBI 2002 Fish Welfare. Briefing Paper 2. Fisheries Society of the British Isles, Granta Information Systems, 82A High Street, Sawston, Cambridge, UK

Huntingford FA, Wright PJ and Tierney JF 1994 Adaptive variation in antipredator behaviour in threespine stickleback. In: Bell MA and Foster SA (eds) *The Evolutionary Biology of the Threespine Stickleback* pp 278-296. Oxford University Press: Oxford, UK

https://doi.org/10.1017/S0962728600030517 Published online by Cambridge University Press

Mikheev V, Metcalfe NB, Huntingford FA and Thorpe JE 1994 Size-related differences in behaviour and spatial distribution of juvenile Atlantic salmon in a novel environment. *Journal of Fish Biology 45*: 379-386

Peichel CL, Nereng KS, Ohgi KA, Cole BL, Colosimo PF, Buerkle CA, Schluter D and Kingsley DM 2001 The genetic architecture of divergence between threespine stickleback species. *Nature 414*: 901-905

Peichel CL, Ross JA, Matson CK, Dickson M, Grimwood J, Schmutz J, Myers RM, Mori S, Schluter D and Kingsley DM 2004 The master sex-determination locus in threespine stickleback is on a nascent Y chromosome. *Current Biology 14*: 1416-1424

Quinn GP and Keough MJ 2002 *Experimental Design and Data Analysis for Biologists*. Cambridge University Press: Cambridge, UK

Rose JD 2002 The neurobehavioural nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science 10*: 1-38

Ross LG and Ross BR 1999 *Anaesthetic and Sedative Techniques for Aquatic Animals*. Blackwell Science: Oxford, UK

Russell NR and Wootton RJ 1992 Appetite and growth compensation in the European minnow, *Phoxinus phoxinus* (Cyprinidae) following short periods of deprivation. *Environmental Biology of Fishes 34*: 275-285

Skov C and Koed A 2004 Habitat use of 0+ year pike in experimental ponds in relation to cannibalism, zooplankton, water transparency and habitat complexity. *Journal of Fish Biology 64*: 448-459

Sneddon LU 2004 Evolution of nociception in vertebrates: comparative analysis of lower vertebrates. *Brain Research Reviews 46*: 123-130

Sneddon LU, Braithwaite VA and Gentle MJ 2003 Do fish have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society of London Series B, Biological Sciences 270*: 1115-1121

Winfield IJ and Nelson JS 1991 *Cyprinid Fishes Systematics, Biology and Exploitation*. Chapman & Hall: London, UK

Wootton RJ 1976 *The Biology of the Sticklebacks*. Academic Press: London, UK

Wootton RJ 1998 *Ecology of Teleost Fishes*, 2nd Edition. Kluwer: Dordrecht, The Netherlands

Wright HA, Wootton RJ and Barber I 2004 Inter-population variation in early growth of three-spined sticklebacks (*Gasterosteus aculeatus* L) under laboratory conditions. *Canadian Journal of Fisheries and Aquatic Sciences 61*:1832-1838

https://doi.org/10.1017/S0962728600030517 Published online by Cambridge University Press