BRIEF COMMUNICATION

Behavioural salinity preference of juvenile yellow perch *Perca flavescens*

E. A. F. Christensen*† and M. Grosell‡

Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark and ‡Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149, U.S.A.

The present study determined the behavioural salinity preference of a freshwater stock of juvenile yellow perch *Perca flavescens* acclimated to salinities of 0 and 10. The preferred salinities ranged between 7·3 and 13·0 (mean ± S.D. = 10·4 ± 1·7; $n = 13$) with no significant effect of acclimation salinity. The results showed that juvenile *P. flavescens* prefers near isoosmotic salinities, which could be due to a lowered energetic cost of osmoregulation.

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Fish live at salinities ranging from 0 in fresh water to >30 in seawater and deal with large temporal and spatial salinity variations in estuaries and tidal ponds (Feldmeth & Waggoner, 1972). Water salinity has been shown to affect the physiological performance of teleosts (*e.g.* growth, metabolism and swimming; Febry & Lutz, 1987; Bœuf & Payan, 2001; Ern *et al.,* 2014), their activity level (Swanson, 1998), their survival (Behrens *et al.,* 2017) and determine distribution patterns of fish species in nature (Serafy *et al.,* 1997).

Environmental salinity exerts osmotic stress on fish as teleosts must keep their blood plasma osmolality around 3–400 mOsm kg⁻¹ (corresponding to a salinity around 10) to maintain homeostasis, yet are permeable to the environment (Brett, 1979; Evans *et al.,* 2005). It is a general assumption that salinities of near isoosmotic levels are beneficial for fish due to a minimized energetic cost of osmoregulation. However, studies on whole-animal energetics in relation to salinity have not necessarily shown optimal salinities at near-isoosmotic conditions (Bœuf & Payan, 2001; Ern *et al.,* 2014). The optimal salinity varies considerably among both stenohaline and euryhaline species, indicating that other factors than ion-regulation, such as hormone levels and hormonal control in relation to osmoregulation (Morgan & Iwama, 1996), can influence the whole-animal energetic response to salinity.

†Author to whom correspondence should be addressed. Tel.: +45 51 22 01 20; email: emil.flindt@bio.ku.dk

Compared with studies on the effect of salinity on whole-animal energetics, only few studies have concerned the behavioural salinity preference of fish in laboratory settings where salinity has been set as the only varying factor (McInerney, 1964; Serrano *et al.,* 2010; Bucking *et al.,* 2012). Most teleosts have only limited scope for coping with varying salinities (Evans, 1984) and behavioural salinity preference could differ among species. The yellow perch *Perca flavescens* (Mitchill 1814) (Thorpe, 1977) is a North American freshwater fish that occurs in brackish water at salinities up to 12 (Keup & Bayless, 1964). It was the aim of the present study to determine the salinity preference, as well as the effect of acclimation salinity on salinity preference, of *P. flavescens* in a laboratory setting.

Animal care and experimental protocols followed the guidelines of and were permitted by, the Institutional Animal Care and Use Committee at the University of Miami (protocol number 13-225).

Juvenile *P. flavescens* (mean $\pm$ s.d. body mass $= 9 \cdot 0 \pm 2 \cdot 4$ g, $n = 13$ and standard length $8 \cdot 8 \pm 0 \cdot 6$ cm) from a freshwater stock were obtained from the freshwater hatchery Northeastern Aquatics (Rhinebeck, NY) and transported to the Rosenstiel School of Marine and Atmospheric Science, Miami, FL, U.S.A. within 24 h. The fish were held in several 15 l aerated aquaria with salinities of either 0 or 10 at 22 °C, at a maximum density of four fish per aquarium. The lighting scheme was kept at 16 h light, 8 h dark. The fish were acclimated for 2·5 months before experimentation began, during which they were fed five times a week with commercial fish pellets. After feeding, 60% of the water was exchanged in each tank. Experiments were conducted during two consecutive weeks and fish were fasted 4 days prior to experimentation.

The salinity preference setup was placed in a temperature-controlled room (22 °C). Visual disturbance was avoided by shielding the experimental aquaria with black sheeting. The experimental aquaria consisted of two 50 cm diameter circular choice tanks, interconnected by a 7 cm wide and 10 cm long junction (Fig. 1). The water depth in the choice tanks was 10 cm. Each choice tank had an adjacent mixing tank of approximately 4 l through which the water circulated at a rate of $1 \, \mathrm{l \, min^{-1}}$, allowing aeration and mixing of the water. The total volume of the preference tanks was 48 l. Salinity could be changed by dosing either saline (filtered sea water from Bay of Biscayne; salinity 33) or fresh water (dechlorinated tap water; salinity 0·4) to the mixing tanks from two 8 l reservoir tanks at rates of $1 \, \mathrm{l \, min^{-1}}$. A water outlet was placed in the mixing tanks. The reservoir tanks were constantly fed with new water at a rate of *c*. $1 \, \mathrm{l \, min^{-1}}$. The temperature in the reservoir tanks were controlled with a relay (PR-5714, PR Electronics: www.preletctronics.dk) and the temperature difference between the choice tanks never exceeded 0·2 °C. All flow rates were monitored and adjusted with clamps before each experiment.

Salinities were measured in the mixing tanks with salinity meters (Cond 3310, WTW: www.wtw.com) and the choice tanks monitored with a USB 2·0 camera (Microsoft LifeCam Cinema USB, Microsoft: www.microsoft.com). The camera and the salinity meters were connected to a laptop PC, the latter through galvanic isolators (USB2ISO, Electronics shop; www.electronics-shop.dk) to prevent electric interference in the salinity measurements. A relay (DAQ-M, Loligo; www.loligosystems .com) for the four dosage pumps was also connected to the PC. In the experimental determination of environmental preference or avoidance, unevenly distributed light can act as a competing factor to the primary factor (Scherer & McNicol, 1998). To
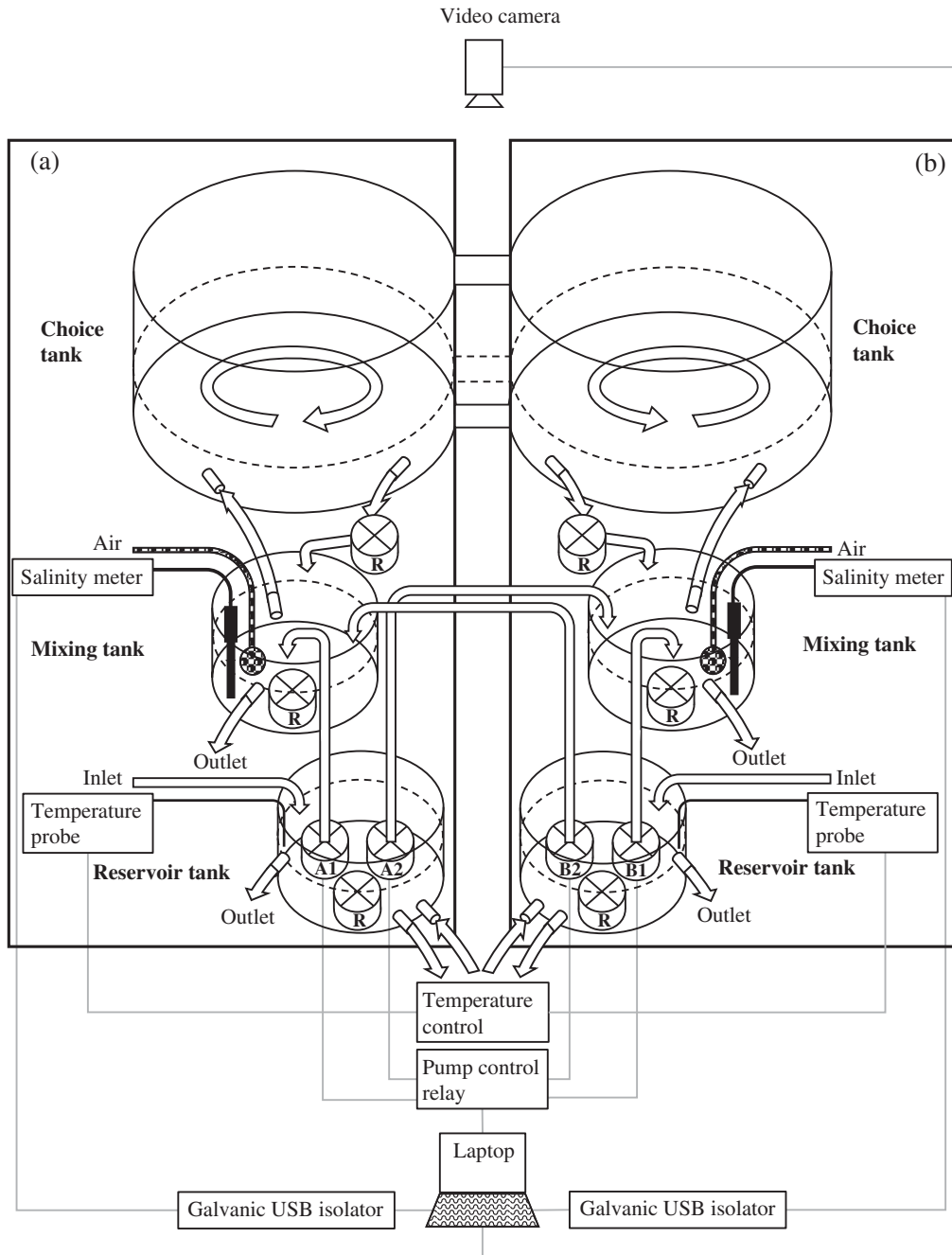
Video camera

(a)

(b)

Choice tank

Choice tank

Air

Air

Salinity meter

Salinity meter

R

R

Mixing tank

Mixing tank

R

R

Outlet

Outlet

Inlet

Inlet

Temperature probe

Temperature probe

Reservoir tank

A1 A2

B2 B1

Reservoir tank

Outlet

R

R

Outlet

Temperature control

Pump control relay

Laptop

Galvanic USB isolator

Galvanic USB isolator

Fig. 1. Diagram of the salinity preference tanks. The fish was only present in the choice tanks (a and b) and could shuttle between them through a narrow junction. R, Recirculation pump; A1, A2, B1, and B2, individually controllable pumps. ⇒, Water flow and direction; _ _ _, water levels; ——, cables.

avoid this, the lighting of the experimental arena was adjusted until a binary contrast filter on the video recordings showed even light distribution.

Each fish was transferred to the experimental tank in a water-filled plastic box and gently placed in the choice tank. Experimental control and data acquisition was accomplished using ShuttleSoft software (Loligo) that monitored the position of the fish and the salinities once s^{-1} *via* the video camera. Each choice tank was predefined

to onset either increasing or decreasing salinity, by activating the dosage pumps in the reservoir tanks in response to presence of the fish in the specific tank: If the fish was in the low salinity choice tank, the salinity of both choice tanks would decrease until the fish moved to the high salinity choice tank after which salinity would increase in both choice tanks. As the dosage pumps delivered water at a constant rate to a preference setup of a constant volume, the salinity changed asymptotically towards the salinity of either seawater or fresh water due to wash-in–wash-out mechanisms (Svendsen *et al.,* 2016). If the fish were in the junction in-between the choice tanks, the salinity would remain stable. The hysteresis was set to a salinity of 0·2 to allow time for the water to mix and a salinity difference of 3 was kept at all times between the two choice tanks. Personal observations as well as studies on the sister species the European perch *Perca fluviatilis* L. 1758 showed that *P. flavescens* did not tolerate salinities of >13–15 for extended periods (Lutz, 1972; Overton *et al.,* 2008). Therefore, the system was set not to exceed a salinity of 15 in the choice tanks to avoid adverse conditions for the fish. To account for potential side bias, the increasing and decreasing sides were switched between experiments. The system salinity was held steady at the acclimation salinity for the first 3 to 4 h of an experiment for the fish to acclimate. After this, the system was set to adjust the salinity according to the position of the fish, as described above, for the next 18 to 20 h.

For each individual, the salinity preference was calculated as the median of the salinities at which the individual was present throughout the experiment (Schurmann and Steffensen, 1992), excluding the first 3 h of acclimation. The data were tested for normality using Shapiro-Wilk's tests and for variance homogeneity with Levene's tests. The effect of acclimation salinity on mean salinity preference of each salinity treatment was tested with a general linear model (GLM) including side setup as a covariate. All statistics were performed in SPSS 24 (IBM: www.ibm.com) with a significance level of $P < 0.05$. The residence time in each choice tank and the junction in-between was calculated from after the initial acclimation to the setup until the experiment ended.

The salinity preferences were normally distributed within both acclimation salinity (Shapiro-Wilk's tests, $P > 0.05$) and there was variance homogeneity (Levene's test, $P > 0.05$) in the salinity preferences between the two treatments. The mean s.D. salinity preference of *P. flavescens* was 9.7 ± 1.6 ($n = 7$) for the fish acclimated to a salinity of 0, while 11.1 ± 1.6 ($n = 6$) for the fish acclimated to a salinity of 10, but no significant effect of acclimation salinity (GLM, $F_{1,12} = 2.128$, $P > 0.05$). The overall mean $\pm$ s.D. salinity preference was 10.4 ± 1.7 ($n = 13$). There was no effect of side setup (GLM, $F_{1,12} = 0.344$, $P > 0.05$).

The residence times in each compartment varied between individuals, as seen on the large standard deviations in Table I: some individuals spent most time in shuttling back and forth between the tanks, while others used the stable salinity situation initiated by residing in the junction between the two tanks to a large extent. The fish resided on average around $^1/_3$ of the time in each choice tank and $^1/_3$ in junction between the tanks.

Perca flavescens occurs in brackish water at salinities up to 12, yet Keup & Bayless (1964) found the highest abundance at salinities below 3·5. In the present study, *P. flavescens* preferred ambient salinities around 10 and the *in situ* observations of salinity preference might be influenced by other habitat factors than salinity alone, such as food availability, predation pressure, temperature, oxygen level and substrate for shelter. The preferred ambient salinities were near iso-osmotic levels, where the fish

TABLE I. Mean ± s.d. per cent residence time over a period of 18–20 h in each choice tank and the junction between the tanks during salinity preference experiments on *Perca flavescens*

Salinity	n	Increasing salinity (%)	Decreasing salinity (%)	Junction−stable salinity (%)
0	7	33 ± 13	44 ± 11	23 ± 19
10	6	33 ± 14	34 ± 22	33 ± 31

probably benefit energetically from a lowered cost of osmoregulation. It has been suggested that optimal salinity is connected with the salinity from which the animals or stock originates (Ern *et al.,* 2014). Probably, this is not the case for *P. flavescens* since the experimental animals in the present study originated from a freshwater stock. *Perca flavescens* also have anadromous ancestry (Victoria *et al.,* 1992), which could enable them to cope readily with salinity changes within their limited tolerance. The results are in accordance with a study on the *P. fluviatilis*, a closely related sister species to *P. flavescens* (Stepien *et al.,* 2016), which showed a significantly decreased aerobic scope in fresh water, compared with a salinity of 10 (Christensen *et al.,* 2017). The reduced aerobic scope observed in freshwater acclimated *P. fluviatilis* suggested elevated metabolic demands associated with osmoregulation. In contrast, Overton *et al.* (2008) found significantly decreased growth in *P. fluviatilis* at salinities above 4 and in Ern *et al.* (2014), the standard metabolic rate of *P. fluviatilis* increased with salinity. This suggests plasticity in the salinity response within the *Perca* L. 1758 genus, or a discrepancy among methodologies.

Behavioural temperature preference can be affected by acclimation temperature in the short term before reaching the final temperature preference in the long term (Reynolds & Casterlin, 1979). In the present study, the salinity preferences of *P. flavescens* was not significantly affected by acclimation to different salinities for 2·5 months. The average salinity preference was, however, slightly lower when the fish had been acclimated to fresh water. Increasing the sample size and the numbers of acclimation salinities could elicit valuable information on short-term salinity preference, especially when studying species with very wide salinity tolerances. However, as the average salinity preference of all individuals in the present study was within a narrow range (7·3–13), increasing sample size would probably not have change the overall result.

Schurmann & Steffensen (1992) showed a skewed proportional residence time in each side of a similar setup to determine temperature preference of *Gadus morhua* L. 1758, which showed that the derived preferred temperature was not an effect of random movement between the tanks. It is, unfortunately, not possible to use the same logic with the setup of the current study since the fish had an option of stable salinity in the junction between the two tanks. In the present study, the varying residence times in each compartment possibly depicts different individuals' strategies to obtain and maintain the preferred salinity. Furthermore, temperature preference of the *P. fluviatilis* in the same setup as Schurmann & Steffensen (1992) also showed skewed proportional residence time in the two tanks (E. A. F. Christensen & J. F. Steffensen, unpubl. data). As *P. flavescens* and *P. fluviatilis* acts very similar to laboratory conditions (personal experience), it is fair to assume that they have similar responses to new environments,

such as a preference setup and that the salinity preference reported in the present study was not a result of random behaviour.

On a note of caution, the current methodology for determining salinity preference used large amounts of water due to the water exchange during salinity changes. The dosage pumps in the present study could potentially deliver $1\,l\,min^{-1}$ throughout a 20 h experiment, that is, $1\,l\,min^{-1}$ x 60 min x 20 h = 1200 l. If constant supply of water had not been available, two reservoir tanks of 1200 l water, one with saline and one with fresh water, would have been necessary for one experiment. Furthermore, the temperature would need to be controlled tightly, as in the present study, in order not to impose temperature as a covariate. Therefore, utilizing as small choice tanks as possible and keeping dosage flow low is recommended whenever possible. For some species, this means limiting the life stage to juveniles.

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