JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 39, No. 6
December, 2008

Interactive Effects of Different Temperatures and Salinities on Growth, Feed Conversion Efficiency, and Blood Physiology in Juvenile Spotted Wolffish, *Anarhichas minor* Olafsen

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Abstract

The aim of this study was to investigate the effects of different salinities and temperatures and their possible interactive effect on growth performance, feeding parameters, and blood physiology in juvenile spotted wolffish, *Anarhichas minor*, reared at different temperature (7 and 10 C) and salinity (15, 25, and 34‰) combinations. There was a significant interactive effect between temperature and salinity on growth, as a growth-enhancing effect was seen at intermediate and full salinities at higher temperature, whereas the reciprocal trend was seen at lower temperature. Mean total feed consumption, daily feeding rate, and feed conversion efficiency were all highest at the intermediate salinity at 10 C, whereas at 7 C, the feeding parameters were highest at low and intermediate salinities. Blood plasma sodium content was lowest at 15‰, whereas the opposite trend was seen in partial pressure of CO_2 and bicarbonate in blood where the highest concentrations were seen at 15‰. This study demonstrates that spotted wolffish has a high osmoregulatory and acclimatory capacity. In an aquaculture context, growth of juvenile spotted wolffish can be improved by rearing the species at high temperature and intermediate salinity combinations at least in a limited period of the juvenile phase.

Teleost fishes regulate their plasma ions such that the internal osmotic pressure of their body fluids is equivalent to approximately 10–15‰ salinity (Brett 1979). It has been proposed that the energy used for the metabolic cost of ionic and osmotic regulation (Brett 1979; Jobling 1994) is reduced in isoosmotic (10–15‰) environments and that these energy savings are translated into growth enhancement (Lambert et al. 1994; Woo and Kelly 1995; Dutil et al. 1997; Imsland et al. 2001). Optimal salinity for growth in marine fish is species specific and also differs with life-history stage and season (Gutt 1985; Morgan and Iwama 1991; Deacon and Hecht 1999). In addition to influencing growth in marine fish, it has also been shown that increased salinity may cause a decrease in the digestibility (protein and feed conversion efficiency [FCE]) (Ferraris et al. 1986; Deacon and Hecht 1999).

The spotted wolffish, *Anarhichas minor* (Olafsen), with its tasty flesh and valuable skin, is a new and promising candidate for the intensive cold water aquaculture industry (Falk-Petersen et al. 1999; Le François et al. 2002; Foss et al. 2004; Sund and Falk-Petersen 2005; Imsland et al. 2006). Spotted wolffish can be described as a typical stenohaline marine cold water teleost, living in depths with small fluctuations in both temperature and salinity (Barsukov 1959). However, in studies on both spotted wolffish (Foss et al. 2001) and common wolffish, *Anarhichas lupus* (L.) (Le François et al. 2004), it has been shown that wolffish have a very

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good osmoregulatory capacity and therefore can be reared at a wide range of salinities. Spotted wolffish may thus be characterized as a euryhaline species. However, the interactive effects of temperature and salinity on growth in spotted wolffish has not been investigated. In summer flounder, *Paralichthys dentatus* (L.), a significant temperature–salinity interaction on growth was seen at high temperatures and salinities (Malloy and Targett 1991). In a study on turbot, *Scophthalmus maximus* (Rafinesque), Imsland et al. (2001) found an interactive temperature–salinity effect as growth and FCE increased with decreasing salinities at high temperatures (18 and 22 C). Hence, the main objective of the present study was to test whether growth of juvenile spotted wolffish could be improved when reared at salinities lower than full-strength seawater and to investigate whether temperature interacts with salinity in relation to growth physiology. Furthermore, it was investigated if the temperature–salinity interaction influenced FCE and osmoregulatory performance. We included monitoring of blood physiological response to investigate if, and to what extent, the hydromineral and acid–base status (sodium, chloride, osmolality, pCO_2, bicarbonate) of spotted wolffish was affected by progressive changes in temperature and salinity.

Materials and Methods

Preexperimental Protocol

The juvenile spotted wolffish used for this study were spawned and hatched at Tomma Marinfisk at the island of Tomma (Nordland County, North Norway). After hatching in April 2005, the juveniles were fed with a commercial formulated feed (Wean-Ex and Dan-Ex 0.8–1.8 mm). On October 13, 2005, the juveniles were transferred to the Industrial Laboratory at the Bergen High Technology Centre (BHTC). After arrival, the fish ($N = 574$) were reared at 8–9 C until the start of the experiment. The experimental tanks were square (1×1 m with rounded corners), grey fiberglass units covered with a plastic lid. The tanks had a rearing volume of 400 L. After arrival to the BHTC, the juveniles were introduced to a new type of commercial formulated feed (containing 52% protein, 18% fat, and 11.9% carbohydrate; T. Skretting A/S, Stavanger, Norway). Pellet size was 1.8 mm before the start of the experiment and 3 mm throughout the experiment. During the experiment, only floating pellets were used. A circular automatic feeder and a fluorescent spotlight (30 W with 7% light intensity) were built into the center of each tank cover. Photon irradiance was measured to be approximately 2.7 μmol/m²/sec in the center at the bottom of the tanks. Initial flow rate in each rearing tank was set to 5–6 L/min and was increased up to 7 8 L/min on December 9. Oxygen saturation was measured weekly during the experiment using a portable OxyGuard Handy Gamma instrument (OxyGuard A/S, Birkerød, Denmark). The oxygen saturation in every tank stayed above 90% throughout the experiment. A constant photoperiod of 18 hours light (2 hours twilight) and 6 hours darkness (light : dark, 18:6) was used throughout the experimental period.

Experimental Design

The experiment was carried out at the BHTC in the period from November 17, 2005, to January 25, 2006. On October 25, 2005, a total of 234 spotted wolffish were randomly chosen, anaesthetized (metacain 0.05 g/L), and tagged intraperitoneally with Trovan® Passive Transponder tags (BTS, Scandinavia, Åhus, Sweden). These tags were used to monitor individual growth rates, condition factor, and length. The total number of juvenile spotted wolffish (tagged and untagged) at the start of the experiment was 574 (47–48 in each tank). On November 16, the fish were gradually acclimated to two different temperatures (7 and 10 C) and three different salinities (15, 25, and 34‰), with each group consisting of two replicate tanks. To obtain the different combinations of salinities and temperatures used in the study, the following water supply was used: freshwater (temperature about 5 C) and seawater (temperature about 8 and 20 C [heated seawater] and salinity of approximately 34‰). Water for each temperature × salinity combination was filtrated (particle and ultraviolet filter), mixed, thoroughly aerated, and led into a header tank from which

it was distributed into the appropriate rearing tanks. Temperature and salinity were measured daily and adjusted accordingly and remained within (SD) 0.2 C and 0.3‰ of that prescribed.

The spotted wolffish were fed to satiation twice a day (in the morning between 0800 and 1000 h and in the afternoon between 1400 and 1600 h using automatic feeders. In order to measure feed consumption, uneaten feed was collected in sieves beneath the outlet and counted (number of feed pellets) half an hour after each feeding period.

Growth Data

To obtain growth data, all individually tagged spotted wolffish in each tank were weighed to the nearest 0.1 g on November 17 and then every 3–4 wk, that is, December 8, December 29, and January 25. During each measurement, the weight of the untagged fish in each group was recorded so that the total biomass in each tank could be calculated. All fish were starved for 24 h before each sampling. Specific growth rate (SGR) of the individually tagged fish was calculated according to the following formula: $\text{SGR} = (e^g - 1) \times 100$, where $g = (\ln(W_2) - \ln(W_1))/(t_2 - t_1)$, W_2 and W_1 are mean weights at days t_2 and t_1, respectively.

Feed Data

Total feed consumption (C_F) was calculated as total feed supplied − total remaining feed in the effluent water. The C_F was calculated on a daily basis and then summarized for each experimental period. FCE was calculated as biomass gain per weight unit of consumed feed, that is, $\text{FCE} = (B_2 - B_1)/C_F$, where C_F is the total feed consumption (g dry matter) in the period and B_1 and B_2 are fish biomass (g wet weight) on days t_1 (start) and t_2 (final), respectively.

Blood Physiology

On November 16 before distribution of the fish to 12 tanks, blood samples were taken from 10 wolffish in order to have initial control values. On December 7, December 28, and January 24, blood samples from four spotted wolffish juveniles were taken from each tank, that is, eight fish from each temperature × salinity combination (total of 48 fish at each date). The fish were anaesthetized (metacain 0.05 g/L) and blood collected from the caudal vessels. To assert chloride concentration and osmolality levels in plasma, blood (0.1 1.0 mL) was extracted from the caudal vessels into heparin-treated syringes and then put into 1.5-mL microcentrifuge tubes kept in ice. Plasma chloride levels were determined using a chloride titrator (CMT 10 chloride titrator; Radiometer, Copenhagen, Denmark). Osmolality was measured using a cryoscopic osometer (Osomat 030; Gonotec GmbH, Berlin, Germany). To monitor possible effect of different rearing temperatures and salinities on general blood physiology, blood was analyzed using an i-STAT Portable Clinical Analyzer (Abbott Inc., Abbott Park, IL, USA). The analyzer was used in conjunction with EC8+ disposable cartridges, measuring blood sodium content and partial pressure of CO_2 (pCO_2) and displaying calculated values of blood bicarbonate content (HCO_3^-).

Statistical Methods

All statistical analyses were carried out using the program Statistica 7.0. Normality was evaluated using Kolmogorov–Smirnov test, and homogeneity of variance was tested using the Levenes's *F*-test. To calculate the effect of different temperature and salinity combinations on weight, SGR, feeding parameters, and blood physiology, a three-way nested ANOVA (Zar 1984) was applied where replicates (random) were nested within temperatures and salinities (fixed). Significant ANOVAs were followed by a two-way ANOVA to investigate the possible interaction between temperature and salinity, and a Student–Newman–Keuls multiple comparison test carried out to locate differences between experimental groups (Zar 1984). A significance level (α) of 0.05 was used if not stated otherwise.

Results

Mortality

During the experiment, a total of five spotted wolffish were found dead. Of these five fish, three were tagged and two untagged. This

amounts to a total mortality of 0.9%. No systematic effect was seen in mortality.

Growth

The initial mean weight ± SD was 22.7 ± 4.7 g and did not differ between the six experimental groups (three-way nested ANOVA, $P > 0.3$; Table 1). The final mean weights at 10 C were significantly higher than the mean weights at 7 C (Student–Newman–Keuls test, $P < 0.05$; Table 1), whereas only minor differences between the salinity groups were seen. However, at the final sampling date, a significant interaction between temperature and salinity was found ($P < 0.05$; Table 1) as the salinity groups showed temperature-dependent response. During the experimental period, SGR varied among temperatures, with fish reared at 10 C displaying the highest growth rates throughout the experimental period (three-way nested ANOVA, $P < 0.05$; Fig. 1). When growth was studied for each temperature separately, growth rate also varied between the salinities. At 10 C, the groups at 25 and 34‰ salinity displayed the highest growth, whereas at 7 C, growth rates were lowest in group at 34‰ salinity. Overall, there were significant differences in growth rate among the different salinities as 15‰ had the lowest overall growth rate at 10 C, but 34‰ displayed the lowest overall growth rate at 7 C where no differences were found between 15 and 25‰. Accordingly, an overall significant interaction between temperature and salinity was found (two-way ANOVA, $P < 0.05$; Fig. 1).

Feed Intake and FCE

In general, feeding parameters were affected by both temperature and salinity. The groups at 25 and 34‰ salinity at 10 C had higher mean feed consumption than the groups at 15 and 34 salinity at 7 C (three-way nested ANOVA, $P < 0.05$; Table 2). Furthermore, the daily feeding rate was significantly higher at 10 C compared to 7 C ($P < 0.05$). FCE was higher in the low and intermediate salinity groups at 10 C compared to the full-strength seawater group at 7 C. Examining the two temperatures separately, salinity did not have a significant effect ($P > 0.05$) on mean feed consumption and FCE (Student–Newman–Keuls test, $P < 0.05$; Table 2). The daily feeding rate was significantly different among salinities at 10 C, with the groups at 25 and 34‰ salinity displaying higher daily feeding rates compared to group at 15‰ salinity. No interactive effect of temperature and salinity on the feeding parameters were found, although the daily feeding rate tended toward significance ($P = 0.07$).

Blood Physiology

The temperature–salinity rearing conditions only had a minor effect on measured blood parameters (Table 3). In January, the sodium content in the blood was highest at 7 C ($P < 0.05$; Table 3). A decrease in plasma chloride concentration with decreasing salinity was seen in December (Student–Newman–Keuls test, $P < 0.05$; Table 3). At the last sampling date, a significant interaction between temperature and salinity on the pCO_2 content was found

TABLE 1. *Mean weight (g) of juvenile spotted wolffish reared at two temperatures and three salinities.*[1]

Temperature (C)	Salinity (‰)	*n*	Date			
			November 17	December 8	December 29	January 25
7	15	39	23.9 (5.6)	31.5 (8.3)	39.3 (11.3)[a]	49.8 (16.4)[b]
	25	39	23.0 (4.9)	30.6 (7.9)	40.3 (11.6)[a]	53.1 (17.3)[b]
	34	37	22.1 (5.4)	28.3 (9.1)	33.8 (13.0)[b]	43.6 (19.6)[b]
10	15	39	23.4 (4.5)	33.2 (7.0)	45.4 (10.5)[a]	64.0 (19.5)[a]
	25	40	21.7 (4.5)	31.8 (6.9)	46.6 (11.1)[a]	69.5 (18.5)[a]
	34	40	21.5 (4.5)	31.8 (7.9)	46.1 (10.8)[a]	72.5 (16.3)[a]

[1] Results are given as mean (SD); *n* denotes the number of fish in each experimental group. All results consist of two replicate tanks. Different superscript letters denote significant differences (Student–Newman–Keuls multiple comparisons, $P < 0.05$) between experimental groups at the same date.

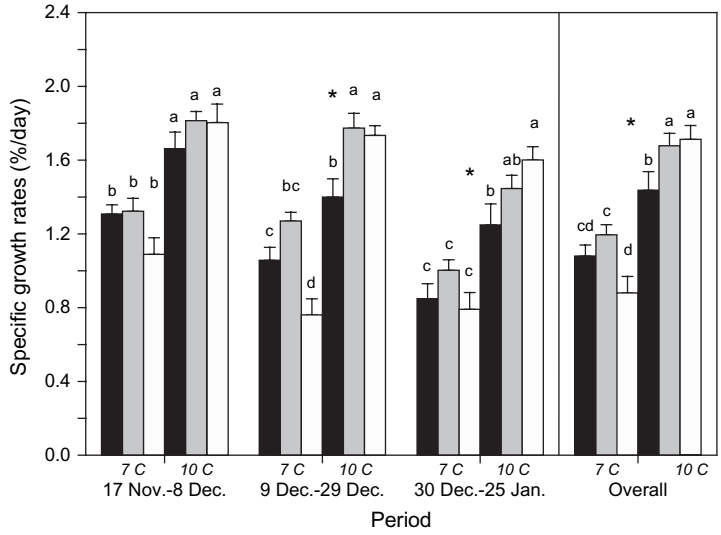

FIGURE 1. *Mean specific growth rates of individually tagged spotted wolffish reared at two temperature and three salinity regimens during the experimental period. Vertical whiskers indicate SE. Different letters denote significant differences (Student–Newman–Keuls test,* $P < 0.05$*) between experimental groups in each period. The symbol "*" indicates significant interaction between temperature and salinity. Bars: black, 15‰; grey, 25‰; and white, 34‰. Bars to left, 7 C; bars to right, 10 C.*

(two-way ANOVA, $P < 0.05$). In general, blood bicarbonate values increased with decreasing temperature and salinity (Student–Newman–Keuls test, $P < 0.05$; Table 3). Throughout the experimental period, wolffish reared at low temperature (7 C) and low salinity (15‰) had significantly higher bicarbonate blood concentrations than those reared at high temperature (10 C) and intermediate (25‰) and high (34‰) salinities (Student–Newman–Keuls test, $P < 0.05$; Table 3).

Discussion

The significant interactive effect between temperature and salinity on growth in the present study is previously unreported for spotted wolffish. In other species, interactive temperature–salinity effects have previously been found, for example, in turbot (Imsland et al. 2001); summer flounder (Malloy and Targett 1991); hogchoker, *Trinectes maculatus* (Bloch and Schneider) (Peters and Boyd 1972); Florida red tilapia; *Oreochromis* sp. (Watanabe et al. 1993); and Nile tilapia, *Oreochromis niloticus* (L.) (Likongwe et al. 1996).

In the present study, feed intake and FCE were affected by temperature and, to a less extent, salinity. The growth-enhancing effect seen at higher temperatures and salinities in the present experiment can be explained by a combination of higher feed consumption (C_T) and better feed utilization. The present findings are partly in-line with the findings of Imsland et al. (2001), as the highest feed consumption and FCE values in both studies were found at salinities lower than full-strength seawater, but the highest daily feeding rate was found at 34‰ in the present experiment and at 15 and 33.5‰ in Imsland et al. (2001). Several studies have shown that salinity affects feed intake and FCE (Dendrinos and Thorpe 1985; Gutt 1985; Lambert et al. 1994; Conides et al. 1997; Imsland et al. 2007), but responses differ between fish species. In a recent study where juvenile Atlantic halibut, *Hippoglossus hippoglossus* (L.) (Imsland et al. 2007), were reared at a constant temperature (12 C) and different salinities (15, 25, and 32‰) for 16 wk, daily feeding rate did not vary significantly between the salinity groups, whereas mean feed consumption and FCE were significantly higher at the lowest salinity compared to full-strength seawater. Higher feed consumption and FCE

TABLE 2. *Mean feed consumption (C_F), daily feeding rate (F), and FCE for juvenile spotted wolffish reared at two temperatures (7 and 10 C) and three salinities (15, 25, and 34‰).*[1]

Temperature (C)	Salinity (‰)	C_F (g wet weight)	F (%)	FCE
7	15	258.6 (40.6)^{bc}	0.82 (0.14)^{c}	1.32 (0.22)^{ab}
	25	284.3 (56.9)^{abc}	0.89 (0.08)^{c}	1.29 (0.06)^{ab}
	34	228.0 (43.8)^{c}	0.81 (0.08)^{c}	1.19 (0.16)^{b}
10	15	395.3 (121.6)^{ab}	1.09 (0.06)^{b}	1.40 (0.04)^{a}
	25	438.1 (132.9)^{a}	1.19 (0.08)^{a}	1.41 (0.06)^{a}
	34	424.4 (143.5)^{a}	1.23 (0.05)^{a}	1.37 (0.06)^{ab}

FCE = feed conversion efficiency.

[1] Results are given as mean (SD). $n = 6$ for each temperature and salinity regimen. Different superscript letters denote significant differences (Student–Newman–Keuls test, $P < 0.05$).

have also been reported in Atlantic cod, *Gadus morhua* (L.) (Lambert et al. 1994), and sea bream, *Sparus aurata* (L.) (Conides et al. 1997), reared at reduced salinities. In contrast, European bass, *Dicentrarchus labrax* (L.) (Dendrinos and Thorpe 1985), and juvenile flounder, *Platichthys flesus* (L.) (Gutt 1985), were found to increase their feed intake with increasing salinity.

In the present experiment, plasma chloride and osmolality generally decreased with decreasing salinity at the first two sampling dates, whereas at the last sampling date, no significant differences between the three different salinities were observed. These findings indicate that juvenile spotted wolffish have a strong osmoregulatory and acclimatory capacity and therefore can tolerate a wide range of salinities. The observed plasma chloride and osmolality concentrations had a developmental pattern similar to that seen in the study of Foss et al. (2001).

TABLE 3. *Measured and calculated blood parameters in juvenile spotted wolffish reared at two temperatures (7 and 10 C) and three salinities (15, 25, and 34‰).*[1]

	7 C			10 C		
	15‰	25‰	34‰	15‰	25‰	34‰
December 7						
Na^+ (mmol/L)	155.2 (4.2)	155.8 (3.4)	160.7 (6.6)	154.1 (3.3)	153.9 (3.4)	158.1 (5.5)
Cl^- (mmol/L)	149.0 (3.4)^{cd}	151.9 (5.4)^{bcd}	158.1 (5.1)^{a}	149.8 (3.5)^{cd}	149.2 (2.2)^{d}	155.1 (2.9)^{abc}
Osmolality (mOsmol/kg)	348.8 (5.3)	350.2 (9.4)	356.3 (11.1)	349.1 (4.8)	354.5 (6.5)	356.4 (5.6)
pCO_2 (mmHg)	19.5 (3.2)	16.0 (5.9)	16.5 (3.8)	16.3 (2.4)	14.4 (3.4)	15.0 (3.4)
HCO_3^- (mmol/L)	5.9 (0.7)^{a}	4.8 (0.9)^{b}	4.4 (0.5)^{bc}	4.5 (0.8)^{bc}	3.8 (0.5)^{c}	3.7 (0.6)^{c}
December 28						
Na^+ (mmol/L)	156.1 (4.4)	158.1 (5.0)	159.7 (2.1)	154.7 (3.1)	156.1 (3.4)	156.3 (3.5)
Cl^- (mmol/L)	153.1 (3.8)^{b}	154.9 (4.2)^{b}	162.5 (3.1)^{a}	154.1 (3.8)^{b}	152.9 (3.9)^{b}	159.6 (4.5)^{a}
Osmolality (mOsmol/kg)	349.8 (14.3)^{bcd}	358.2 (6.3)^{abc}	365.6 (10.4)^{a}	343.0 (9.8)^{d}	350.8 (4.4)^{bcd}	354.8 (8.1)^{abcd}
pCO_2 (mmHg)	18.4 (4.8)	19.0 (4.7)	16.2 (4.8)	16.6 (4.1)	15.0 (3.4)	16.4 (5.8)
HCO_3^- (mmol/L)	5.7 (0.8)^{a}	5.3 (2.0)^{ab}	4.6 (0.6)^{ab}	4.9 (1.3)^{ab}	4.0 (0.6)^{b}	3.8 (0.7)^{b}
January 24						
Na^+ (mmol/L)	156.0 (3.0)^{a}	157.0 (2.6)^{a}	158.4 (3.1)^{a}	156.1 (3.6)^{ab}	152.4 (2.2)^{b}	155.0 (2.9)^{ab}
Cl^- (mmol/L)	163.1 (5.3)^{ab}	162.3 (2.8)^{ab}	163.9 (4.6)^{a}	161.2 (4.0)^{ab}	158.0 (1.6)^{b}	160.6 (3.9)^{ab}
Osmolality (mOsmol/kg)	363.3 (14.5)	358.9 (9.3)	359.8 (9.9)	352.9 (7.6)	351.1 (6.1)	351.0 (6.0)
pCO_2 (mmHg)	16.9 (1.6)^{ab}	14.5 (1.1)^{bc}	17.5 (3.6)^{a}	16.5 (2.5)^{abc}	13.2 (0.9)^{c}	13.1 (2.4)^{c}
HCO_3^- (mmol/L)	5.4 (0.8)^{a}	4.4 (0.9)^{ab}	5.2 (1.3)^{a}	4.6 (0.6)^{ab}	4.0 (0.2)^{b}	3.5 (0.5)^{b}

[1] Values are given as mean (SD). Different superscript letters denote significant differences (Student–Newman–Keuls multiple comparisons, $P < 0.05$) between experimental groups at the same date. Initial values measured at November 16 were the following: Na+, 153.9 (6.2); Cl−, 159.6 (3.8); osmolality, 347.0 (8.7); pCO_2, 17.5 (3.8); and HCO_3^-, 4.4 (0.8).

Among other blood parameters measured in the present study, pCO_2 and HCO_3^- were generally higher at the lowest salinity (15‰) compared to full-strength seawater (34‰). Imsland et al. (2007) found the lowest blood pCO_2 and HCO_3^- values and the highest growth in Atlantic halibut at 15‰, which is in contrast to the present findings. However, sodium levels in blood plasma showed the same trend in both these studies, that is, in general, sodium increases with increasing salinity, indicating lower osmotic activity at the isoosmotic environment. Other studies have also demonstrated an increase in sodium levels with increasing salinity, for example, turbot (Gaumet et al. 1995); emperor angel fish, *Pomacanthus imperator* (Woo and Chung 1995); and coho salmon, *Oncorhynchus kisutch* (Morgan and Iwama 1998). Imsland et al. (2007) concluded that rearing juvenile Atlantic halibut at lower salinities resulted in lower levels of osmoregulatory and metabolic activity and further to reduced energy expenditures, which contribute to higher growth rates at lower than full salinity.

Spotted wolffish can clearly live in a wide range of salinities (Foss et al. 2001, 2004; present study). To determine if a species is euryhaline or stenohaline, Woo and Chung (1995) argued that the ecological habitat of the species played an important role. They speculated that the reason for a typical marine fish to be euryhaline can be explained by the evolutionary history of bony fish. In general, earlier forms of marine teleosts were evolving in an environment less saline compared to today. By the definition of Woo and Chung (1995), and the results of the present study and the study of Foss et al. (2001), spotted wolffish qualifies a euryhaline species. This definition is in contrast to Barsukov (1959) who argued that because the spotted wolffish in its natural habitat only experiences minor fluctuations in salinity, the species could not tolerate reduced salinities. Because many marine fish species spend parts of their lives (juvenile) in estuaries and along the coast, Wu and Woo (1983) argued that marine inshore species generally appear to be more euryhaline than deepwater, offshore species. In contrast to their statement, several recent studies on marine fish living their whole life in seawater have concluded that these species can be reared at a wide range of salinities (Woo and Chung 1995; Foss et al. 2001; Imsland et al. 2007; present study).

Conclusions

A significant interactive effect between temperature and salinity on growth in juvenile spotted wolffish was seen, as intermediate and full salinities had growth-enhancing effects at higher temperature, whereas the reciprocal trend was seen at lower temperature. This study has also confirmed that spotted wolffish has a high osmoregulatory capacity, can be reared at a large range of salinities, and may thus be characterized as a euryhaline species.

Acknowledgment

We thank Bjørn Sveinsbø for valuable technical assistance.

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