JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 38, No. 1
March, 2007

Comparison of Juvenile Spotted Wolffish, *Anarhichas minor*, Growth in Shallow Raceways and Circular Tanks

ALBERT K. IMSLAND[1] AND SNORRI GUNNARSSON

Akvaplan-niva, Iceland Office, Akralind 4, 201 Kópavogi, Iceland, and Department of Biology, University of Bergen, High Technology Centre, N-5020 Bergen, Norway

A. FOSS

Akvaplan-niva, Bergen Office, Nordnesboder 5, N-5005 Bergen, Norway

LARS OLAV SPARBOE AND VICTOR ØIESTAD

Akvaplan-niva, Polar Environmental Centre, N-9296 Tromsø, Norway

SINDRI SIGURÐSSON

Hlýri ehf, Hafnarbraut 6, 740 Neskaupstaður, Iceland

Abstract.—We compared growth properties of juvenile spotted wolffish with initial mean weight (±SE) 105.9 (±3.1) g reared in shallow raceways and conventional circular tanks in a 202-d-long growth trial at ambient temperature (approximately 4.0 C). From Day 41 onward, the fish in the shallow raceways was significantly larger, and final mean weights were 356.3 (±18.2) and 318.2 (±15.6), in the shallow raceways and the circular tanks, respectively. Overall, growth rates were 14% higher (0.62%/d) in the shallow raceway group compared to the circular tanks (0.52%/d). Feed conversion efficiency differed and was 17% higher in the shallow raceways (1.01) compared to the circular tanks (0.84). Correlation between adjacent growth rates was more profound in the circular tanks (mean Spearmans rank, r_{Sp} = 0.38) than in the shallow raceways (mean r_{Sp} = 0.15). This could indicate a stronger social hierarchy in conventional tanks leading to suppressed growth, which is in line with the growth data presented in this study. The findings of the present study may have important consequences for optimization of commercial production of spotted wolffish and could be applicable to other bottom-dwelling species.

The idea of using a shallow raceway system (SRS) for fish production appeared in the late 1980s and has been developed in an experimental scale (e.g., Øiestad 1999). A shallow raceway is similar to a standard raceway, with some small, but crucial, differences that must be fully accomplished: (1) low water level, ranging from 0.7 to 25 cm, depending on the fish size; (2) high density, where fish use 200–300% of the available bottom area; and (3) the existence of a turbulent and plug-flow pattern (Øiestad 1999; Foss et al. 2004; Labatut and Olivares 2004). The principle has been demonstrated to work in single raceways without the reuse of water (flow-through once, Øiestad 1999; Foss et al. 2001; Foss and Imsland 2002). The technology is especially well suited for land-based production of flatfish like turbot, *Scophthalmus maximus* (Rafinesque); and halibut, *Hippoglossus hippoglossus* (L.); or bottom-dwelling species like the spotted wolffish, *Anarhichas minor* (Olafsen), as it opens possibilities for reduced land requirement and building investments as well as reduced water consumption, by stacking the raceways in several levels and reusing the water from level to level. The compactness, and the self-cleaning properties of the system in combination with high stocking density, are important characteristics of the SRS. But it is also possible that growth could be improved in the SRS compared to conventional circular tanks because of better utilization of the feed (e.g., pellets floating over a large area could increase each fish possibility of receiving pellet) and lower antagonistic behavior (i.e., less water volume to intimidate conspecifics). In contrast, Hickman and Tait (2001) suggested (using a literature comparison of growth data)

[1] Corresponding author.

that the shallow raceway was a less effective rearing system for New Zealand turbot, *Colistium nudipinnis* (Waite 1910), than conventional tanks. In Norway and Iceland, spotted wolffish are commonly reared in shallow raceways (e.g., Foss et al. 2001, 2002; Imsland et al. 2006), a system designed for carrying high densities of fish. However, no direct comparison between growth properties of candidate species, for example, spotted wolffish, in shallow raceways and circular tanks has been published. Accordingly, the main aim of the present study was to compare growth of juvenile spotted wolffish in shallow raceways and conventional circular tanks.

Many different factors have been put forward to explain observed size variation in an aquaculture fish population; for example, genetic differences producing size variability (Forsberg 1996; Imsland et al. 1998), social interactions (Jobling and Koskela 1996), and antagonistic behavior (McCarthy et al. 1992; Imsland et al. 1998). Social interactions can lead to decreased growth in low-ranking individuals (McCarthy et al. 1992; Jobling and Koskela 1996) and leading to the establishment of size hierarchy. The establishment of size hierarchy may be caused by direct competition for food (McCarthy et al. 1992; Jobling and Koskela 1996) or by other, less well-understood, social interactions (Jobling 1982). It is important to study, and compare, the size-related hierarchies in shallow raceways and conventional tanks as less stringent size hierarchy in either rearing system could lead to more homogenous biomass growth.

The following experiment was designed to investigate the effects of different rearing units on growth rate and feed efficiency ratio in juvenile spotted wolffish. Attempts were made to study possible formation of size hierarchies and social interactions in the two rearing systems. The aim of the study is to compare ingrowth, feeding utilization, and formation of size hierarchies in the two rearing systems.

Materials and Methods

Fish Stock and Rearing Conditions

Spotted wolffish juveniles (see Imsland et al. 2006 for more details on larval and early juvenile phase) were reared in shallow raceways (Øiestad 1999) and fed using commercial dry pellets until the start of the experiment. In November 2005, the fish were distributed randomly into two raceways (0.4 × 1.8 m, 0.72 m^2) with a water level of 8–10 cm, providing a total volume of 0.06–0.08 m^3 (60–80 L) and into two circular tanks (bottom area, 0.78 m^2) with a water level of 5–20 cm (volume 0.04–0.16 m^3, 40–160 L). Hence, the experimental setup is a two-way nested design, where the two replicates are nested within the experimental groups (i.e., shallow raceways and circular tanks).

Water flow was set to 10–15 L/min for each of the experimental units. In the shallow raceways, the water inlet pipe was in front of perforated screens. To avoid the formation of jet currents, the inlet flow was pointed against the wall and away from the screens. In the circular tank, the water inlet was through a vertical inlet pipe approximately 5 cm from the sidewall creating a circular flow movement in the tank. The water current speed during the trial was between 2–3 cm/s in both systems. Oxygen saturation was measured with a hand-held Oxygard Handy Alpha meter (Oxyguard International, Birkenrød, Denmark) at regular intervals in the effluent water of all rearing units and was never below 80%. Two 25-W light bulbs dimmed to 5% of maximum capacity supplied the rearing units with low-level light during the experiment and all groups were maintained under a constant photoperiod of eighteen hours of light and six hours of dark (LD18:6).

Experimental Design

The growth study was carried out from December 1, 2005, to June 21, 2006, with 188 juvenile spotted wolffish. On November 21, 2005, in preparation for the study, a subgroup within each rearing unit (n = 19–21 in each unit, n_{total} = 78) were tagged intraperitoneally with Trovan® Passive Transponder (Trovan Ltd., BTS Scandinavia, A.B. Åhus, Sweden) tags. The fish were reared under an ambient seawater regime for Neskaupstaður with minimum in March (approximately 3.0 C) and maximum in June (4.5 C). The tagged fish were reared together with 109 untagged fish (19 fish in one circular tank, 30 fish in the three other units) giving a stocking density of 3.7–5.1 kg/m^2 in all rearing units at the start of the experiment. All fish were anaesthetized (phenoxyethanol, 0.05 g/L) and weighed individually approximately every 6 wk during the experiment. Growth results are based on registration of the tagged fish and other results are based on tank biomass registrations.

The fish were fed a commercial formulated floating feed (Dan-ex 1562, Dana Feed, Horsens, Denmark) containing 53% protein, 17% fat, and 8% carbohydrate (pellet size 4–6 mm). Feed was provided in excess from automatic belt feeders for 6 h daily (0900–1500 h) situated close to the water inlet and thus enabling the pellets to float downstream. In addition, the fish were hand-fed occasionally to ensure that a satiation level was obtained. Uneaten pellets were collected after each feeding (no later than 30 min after each feeding pulse) and counted to estimate feed intake. The uneaten feed was collected by filtering the outlet water with a fine mesh. Our observations showed that the amount of feed broken down was negligible in the short time from feeding to sampling and that this sampling method gave an accurate estimate of the amount of feed eaten.

Feed Data

Total feed consumption (C_T) was calculated as total feed supplied − total remaining feed in the effluent water. C_T was calculated on a daily basis and then summarized for each of the four rearing units. Daily feeding rate ($F\%$) was calculated as follows:

$$F\% = 100[C/((B_1 + B_2)/2)](t_2 - t_1)^{-1}$$

where C is 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. Feed conversion efficiency (FCE) was calculated as biomass gain per weight unit of consumed feed:

$$\text{FCE} = (B_2 - B_1)/C$$

Data Analysis and Statistical Methods

Specific growth rate (SGR) was calculated according to the formula SGR $= (e^g - 1) \times 100$, where $g = (\ln W_2 - \ln W_1)(t_2 - t_1)^{-1}$ and W_2 and W_1 are wet weights (g) at days t_2 and t_1, respectively. A two-way nested ANOVA (Searle et al. 1992), where the two replicates were nested within rearing systems (i.e., shallow raceways or circular tanks), was applied to calculate the effect of different rearing systems on mean weights and SGR.

The model equation of the nested ANOVA had the form:

$$X_{ijk} = \mu + \alpha_i + B_{ij} + \epsilon_{ijk} \quad (1)$$

where μ is the general level, α_i is the treatment effect of rearing system (i.e., shallow raceways or circular tanks), B_{ij} is the contribution caused by replicate (here tank a or b and raceways a and b) j in group i, and ϵ_{ijk} is the error term. Significant ANOVAs were followed by a Student–Newman–Keuls multiple comparison test (Zar 1984) to locate differences among treatments. For parameters where only group data existed (FCE, $F\%$, and C_T) a two-way nested ANOVA was applied, followed by a Student–Newman–Keuls multiple comparison test to locate any differences among treatments.

Individual growth trajectories were analyzed using a growth curve analysis (GCM) multivariate analysis of variance (MANOVA) model (Timm 1980; Chambers and Miller 1995). The model equation of the GCM had the form:

$$\mathbf{Y}(n \times p) = \mathbf{X}(n \times q)\mathbf{B}(q \times p) + \mathbf{E}(n \times p) \quad (2)$$

where $\mathbf{Y}(n \times p)$ are the growth at age vectors

$$\mathbf{y} = (y_1, y_2, \ldots, y_p) \quad (3)$$

for each p (age) measurements on n individual fish, $\mathbf{X}(n \times q)$ is the design matrix or the set of extraneous variables measured for each individual, that is, q = age$_p$ + rearing system$_i$ + replicate$_j$ (i = shallow raceway or circular tank; k = replicate a, replicate b); $\mathbf{B}(q \times p)$ is the matrix of parameters estimated by the model; and $\mathbf{E}(n \times p)$ is the matrix of deviations for each individual from the expected value of $\mathbf{Y} = \mathbf{XB}$.

Size ranking (initial size rank versus final size rank) and growth rate ranking (initial growth rate versus final growth rate and growth in adjacent periods) was tested using Spearmans rank correlation (r_{Sp}) (Zar 1984). A significance level (α) of 0.05 was used if not stated otherwise. In cases with nonsignificant statistical tests, power ($1 - \beta$) analysis were performed using the PASS program package (Hintze 1996) using $\alpha = 0.05$.

Results

Mortality

During the experiment, a total of six tagged fish were found dead either in the rearing units or on the floor. This amounts to 7.6% mortality. Overall mortality (tagged and untagged fish) was 7.2% (12 fish). No systematic trend was found as mortality occurred in all rearing units and no size difference was found between the dead and the surviving fish.

Growth: Effect of Culture System

The initial mean weight (±SE) was 105.9 (±3.1) g and did not differ significantly between the two rearing systems, that is, shallow raceways and circular tanks (two-way nested ANOVA, power [$1 - \beta > 0.6$]). From Day 41 onward, the mean weights in the two rearing systems varied (two-way nested ANOVA, $P < 0.01$, Fig. 1A) as the fish in the shallow raceways were larger. Final mean weights ± SE were 356.3 ± 18.2 and 318.2 ± 15.6 in the shallow raceways and the circular tanks, respectively. Mean individual growth trajectories were different (GCM, $\text{MANOVA}_{\text{GROUP}}$, Wilk's lambda $(\Lambda)_{5,\,55} = 0.65$, $P < 0.001$) between the shallow raceways and the circular tanks. Significant higher growth rates for the shallow raceways group were found in three experimental periods (Student–Newman–Keuls test, $P < 0.05$, Fig. 1 B). Overall mean growth rates were 0.52 and 0.62%/d for fish in circular tanks and shallow raceways, respectively.

Feed Intake and FCE

Daily feeding rate did not differ in the two groups (Table 1). However, FCE differed between shallow raceways and circular tanks (two-way nested ANOVA, $F_{1,\,14} = 3.7$, $P < 0.05$, Table 1) and was 17% higher in the shallow raceways compared to the circular tanks.

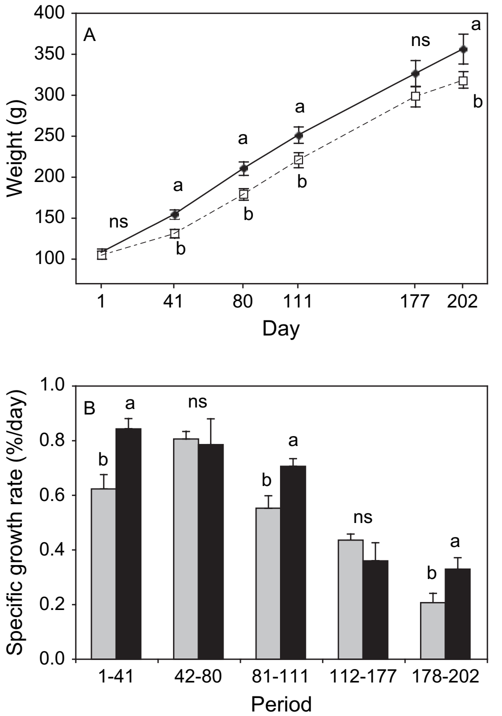

FIGURE 1. *Mean weight (A) and specific growth rates (B) of juvenile spotted wolffish reared in shallow raceways and circular tanks. Vertical line indicating SE may be obscured by symbol. Different letters indicate statistical differences (two-way nested ANOVA,* $P < 0.05$*), with "a" as the highest value. The values for two replicates are combined.* $N = 38–42$ *for each mean value. Symbols in A: shallow raceways, whole line, and filled diamonds; circular tanks, dashed line, and open squares. Symbols in B: shallow raceways, black bars; circular tanks, white bars.*

Size and Growth Ranking

A significant size rank correlation (initial weight versus final weight) was maintained throughout the study in both groups ($r_{Sp} > 0.58$, $P < 0.05$, Table 2) but was higher in the circular tanks ($r_{Sp} = 0.81$). Further, an overall positive correlation between adjacent periods (i.e., period_n and period_{n+i}) growth rates was only found in the circular tanks. When comparing initial versus final growth rates, no correlation

TABLE 1. *Feed consumption (C_T), daily feeding rate (F) and FCE of juvenile spotted wolffish reared in shallow raceways or circular tanks.*

Experimental group	C_T (g ww)	F%	FCE
Shallow raceways	2362.7 (756.9)[a]	0.61 (0.13)	1.01 (0.21)[b]
Circular tanks	1792.7 (520.9)[b]	0.66 (0.12)	0.84 (0.22)[a]

FCE = feed conversion efficiency.

Results are given as arithmetic mean (SD) for the five experimental periods (i.e., $N = 10$ for each experimental group). Different superscripts denote significant differences (Student–Newman–Keuls multiple comparisons, $P < 0.05$) between experimental group.

was found in either group, as initial growth rates were slightly negatively correlated (not significantly) with final growth rates in both groups.

Discussion

Growth rate and feed efficiency ratio of juvenile spotted wolffish was positively influenced by rearing in SRS. Overall growth rate and FCE, respectively, was 14 and 17% higher in the SRS compared to the circular tanks. To the authors' knowledge, there are no published growth studies that directly compare growth of bottom-dwelling species cultured in shallow raceways with conventional circular tanks, but Labatut and Olivares (2004) reared juvenile turbot in SRS and compared their data with existing data in the literature. They concluded that this alternative culture system could produce similar growth rates but at higher densities than in conventional systems. They also found higher FCE for turbot reared in SRS compared to earlier studies conducted in circular tanks. Part of the growth differences found in the present study could be explained by less human intervention into the fish environment because of the less frequent husbandry tasks required in the shallow raceways. Daily tasks in conventional tanks may be stressful to the fish, including sweeping of the bottom area and walls of the tank and flushing almost the entire volume of the tank. Conversely, shallow raceways are self-cleaning and thus minimal human intervention into fish environment is required. Further, they may be attributes facilitating better growth in SRS linked to water transport aspects and swimming behavior. The current pattern in SRS is turbulent, which ensures almost the same current speed everywhere in the tank (Øiestad 1999), whereas current speed in a circular tank will vary with greatest water current speeds near the tank wall (Ross and Watten 1998). The more even current speed in the SRS suits the behavior of spotted wolffish as it seeks very dense aggregations of conspecifics (Foss et al. 2001). In circular tanks, it may find itself swimming against the current near the tank wall spending more energy for swimming than in the SRS. In our study, we observed the highest density of fish away from the tank wall (often near the tank center) in the circular tanks, whereas a more homogenous aggregation was seen in the SRS. This may indicate that spotted wolffish is using less energy for swimming in the SRS allowing more energy to be allocated to growth, which could partly explain the growth differences seen between the two systems in the current study.

TABLE 2. *Results from Spearman rank analysis of correlation for weight and size ranking of individually tagged juvenile spotted wolffish reared in shallow raceways or circular tanks.*

Rearing unit type	Comparison	Spearman rank (r_{Sp})	p for $r_{Sp} = 0$
Shallow raceways	W_1 vs W_6	0.58*	<0.05
	G_1 vs G_2	0.12	>0.35
	G_2 vs G_3	0.18	>0.30
	G_3 vs G_4	0.14	>0.30
	G_4 vs G_5	0.16	>0.30
	G_1 vs G_5	−0.19	>0.25
Circular tanks	W_1 vs W_6	0.81*	<0.001
	G_1 vs G_2	0.44*	<0.05
	G_2 vs G_3	0.56*	<0.05
	G_3 vs G_4	0.16	>0.40
	G_4 vs G_5	0.36*	<0.05
	G_1 vs G_5	−0.25	>0.20

Results are given for size ranking (initial, W_1 *vs* final weight, W_6), growth rank in adjacent periods (i.e., G-period$_n$ and G-period$_{n+i}$), and initial and final growth rank (G_1 vs G_5). Significant rankings are indicated by *.

In our study, there seems to be a growth delay in the circular tank group (first period in Fig. 1 B) as this group might have experienced a higher stress of acclimatization (as both groups were reared in shallow raceways prior to the study). However, this is compensated in the next period as growth increases in the circular group but is slightly reduced in the shallow raceway group. Omitting the first period, there is still 11% difference in overall growth. However, the strength of the study might have been further improved by applying a longer acclimation period.

In general, land-based farming requires more resources for the construction as well as for the fish production compared to production in netpens in the open sea (Oca et al. 2002). For that reason, it is strongly needed to implement technology that is competitive and has the potential to be profitable also in the future. In conventional land-based farming, feed accounts for approximately 30% of the production costs (Oca et al. 2002), so it is important to optimize feeding management. In the present study, a 17% higher FCE was achieved in the shallow raceways. The higher FCE observed in the shallow raceway group in the present experiment may indicate that the turbulent flow in the SRS can lead to a more homogenous feed distribution, which will provide more equal opportunities for all the fish to consume pellets during feeding. The application of shallow raceways in full-scale fish farming is still at an early stage of development, but our findings are encouraging both in terms of fish husbandry and growth management.

The high size rank correlation observed in both experimental groups may indicate an early establishment of stable size ranks (hierarchies), which is common under culture conditions (Huntingford et al. 1990; Imsland et al. 1998). The lack of correlation between initial and final growth rates seen in the present study indicates that there might be a stochastic element causing heterogeneous growth. As growth rate in both experimental groups declined with increasing size (Fig. 1), the lack of correlation between initial and final growth can be interpreted as increased growth variation with increasing size. It is notable that the correlation between adjacent growth rates is more profound in the circular tank. This could indicate a stronger social hierarchy in circular tanks compared to shallow raceways. Social hierarchy may contribute to the observed growth differences between the rearing units as growth can be suppressed by competition under such conditions (Sunde et al. 1998). It has been pointed out by Klokseth and Øiestad (1999) and Øiestad (1999) that less antagonistic behavior can be expected in the SRS compared to other rearing systems. As feed is floating past all individuals in the system, there will be less competition for feed. This could lead to less stringent formation of hierarchies compared to conventional tanks and thereby reducing size variation that is in line with our findings.

In conclusion, the present study indicates that growth and FCE can be improved in spotted wolffish culture by rearing the fish in shallow raceways. These findings may have important consequences for optimization of commercial production of spotted wolffish and could be applicable to other bottom-dwelling species.

Acknowledgment

The present study was financed by the European Commission (contract no. 016869, Raceways).

Literature Cited

Chambers, R. C. and T. J. Miller. 1995. Evaluating fish growth by means of otolith increment analysis: special properties of individual-level longitudinal data. Pages 155–175 *in* D. H. Secor, J. M. Dean, and S. E. Campana, editors. Recent developments in fish otolith research. University of South Carolina Press, Colombia, South Carolina, USA.

Forsberg, O. I. 1996. Optimal stocking and harvesting of size-structured farmed fish: a multi-period linear programming approach. Mathematics and Computers in Simulation 42:299–305.

Foss, A. and A. K. Imsland. 2002. Compensatory growth in the spotted wolffish (*Anarhichas minor* Olafsen) after a period of limited oxygen supply. Aquaculture Research 33:1097–1101.

Foss, A., T. H. Evensen, and V. Øiestad. 2002. Effects of hypoxia and hyperoxia on growth and food conversion efficiency in the spotted wolffish *Anarhichas minor* (Olafsen). Aquaculture Research 33:437–444.

Foss, A., T. H. Evensen, A. K. Imsland, and V. Øiestad. 2001. Effects of reduced salinities on growth, food conversion efficiency and osmoregulatory status in the spotted wolffish. Journal of Fish Biology 59:416–426.

Foss, A., A. K. Imsland, I.-B. Falk-Petersen, and V. Øiestad. 2004. A review of the culture potential of spotted wolffish *Anarhichas minor* Olafsen. Reviews in Fish Biology and Fisheries 14:277–294.

Hickman, R. W. and M. J. Tait. 2001. Experimental rearing of juvenile New Zealand turbot *Colistium nudipinnis* (Waite 1910): a potential new aquaculture species. Aquaculture Research 32:727–737.

Hintze, J. L. 1996. PASS users guide. NCSS, Kaysville, Utah, USA.

Huntingford, F. A., N. B. Metcalfe, J. E. Thorpe, W. D. Graham, and C. E. Adams. 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar* L. Journal of Fish Biology 36:877–881.

Imsland, A. K., T. Nilsen, and A. Folkvord. 1998. Stochastic simulation of size-variation in turbot: possible causes analysed with an individual based model. Journal of Fish Biology 53:237–258.

Imsland, A. K., A. Foss, L. O. Sparboe, and S. Sigurðsson. 2006. The effect of temperature and fish size on growth and food efficiency ratio of juvenile spotted wolffish. Journal of Fish Biology 68:1107–1122.

Jobling, M. 1982. Some observations on the effects of feeding frequency on the food intake and growth of plaice, *Pleuronectes platessa* L. Journal of Fish Biology 20:431–444.

Jobling, M. and J. Koskela. 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. Journal of Fish Biology 49:658–667.

Klokseth, V. H. and V. Øiestad. 1999. Forced settlement of metamorphosing halibut (*Hippoglossus hippoglossus* L.) in shallow raceways: growth pattern, survival and behaviour. Aquaculture 176:117–133.

Labatut, R. A. and J. F. Olivares. 2004. Culture of turbot (*Scophthalmus maximus*) juveniles in shallow raceways tanks and recirculation. Aquacultural Engineering 32:113–127.

McCarthy, I. D., C. G. Carter, and D. F. Houlihan. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology 41:257–263.

Oca, J., L. Reig, and R. Flos. 2002. Is land-based sea bream production a feasible activity on the northwest Mediterranean cost? Analysis of production costs. Aquaculture International 10:29–41.

Øiestad, V. 1999. Shallow raceways as a compact, resource-maximizing farming procedure for marine fish species. Aquaculture Research 20:831–840.

Ross, R. M. and B. J. Watten. 1998. Importance of rearing-unit design and stocking density to the behavior, growth and metabolism of lake trout (*Salvelinus namaycush*). Aquacultural Engineering 19:41–56.

Searle, S. R., G. Casella, and C. E. McCulloch. 1992. Variance components. John Wiley & Sons, New York, New York, USA.

Sunde, L. M., A. K. Imsland, A. Folkvord, and S. O. Stefansson. 1998. Effects of size grading on growth and survival of juvenile turbot reared at two temperatures. Aquaculture International 6:19–32.

Timm, N. H. 1980. Multivariate analysis of variance of repeated measurements. Pages 41–87 *in* P. R. Krishnaiah, editor. Handbook of statistics, vol. 1, Analysis of variance. North-Holland Publishing Company, Amsterdam, The Netherlands.

Zar, J. H. 1984. Biostatistical analysis, 2nd edition. Prentice-Hall, Englewood Cliffs, New Jersey, USA.