The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (<u>http://www.aquaculturehub.org</u>) members and registered individuals and institutions. Please visit our website (<u>http://siamb.org.il</u>) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief

Dan Mires

Editorial Board

Sheenan Harpaz	Agricultural Research Organization Beit Dagan, Israel
Zvi Yaron	Dept. of Zoology Tel Aviv University Tel Aviv, Israel
Angelo Colorni	National Center for Mariculture, IOLR Eilat, Israel
Rina Chakrabarti	Aqua Research Lab Dept. of Zoology University of Delhi
Ingrid Lupatsch	Swansea University Singleton Park, Swansea, UK
Jaap van Rijn	The Hebrew University Faculty of Agriculture Israel
Spencer Malecha	Dept. of Human Nutrition, Food and Animal Sciences University of Hawaii
Daniel Golani	The Hebrew University of Jerusalem Jerusalem, Israel
Emilio Tibaldi	Udine University Udine, Italy

Published under auspices of **The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB), University of Hawaii at Manoa Library** and **University of Hawaii Aquaculture Program** in association with **AquacultureHub** http://www.aquaculturehub.org







ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER: Israeli Journal of Aquaculture - BAMIGDEH -Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL Phone: + 972 52 3965809 <u>http://siamb.org.il</u>

Copy Editor Ellen Rosenberg

EFFECT OF TEMPERATURE ON EMBRYONIC DEVELOPMENT IN SHARPSNOUT SEABREAM (DIPLODUS PUNTAZZO) EGGS

Kursat Firat*, Sahin Saka and Osman Ozden

Department of Aquaculture, Faculty of Fisheries, Ege University, 351000 Bornova, Izmir, Turkey

(Received 10.9.04, Accepted 25.1.05)

Key words: *Diplodus puntazzo*, egg, embryonic development, sharpsnout seabream, temperature

Abstract

Embryonic development of sharpsnout seabream (*Diplodus puntazzo*) was studied at eleven water temperatures (two degree intervals from 12 to 32°C). Embryos successfully developed at temperatures of 16-28°C. Cells did not divide at the temperature extremes of 12 and 32°C. Total mortality was observed by the 128 cleavage stage at 14°C and all eggs were dead by the time the embryos extended 2/3 of the internal circumference of the egg at 30°C. There was an inverse relationship between incubation temperature and the rate of embryonic development.

Introduction

The commercially important sharpsnout seabream (*Diplodus puntazzo*) lives on rocky bottoms of the Mediterranean and Black Seas and the Atlantic Ocean, spawning in September and October (Tortonese, 1970). Its increasing importance as a new aquaculture species in the Mediterranean has interested many scientists in its reproduction and physiology (Georgiou and Stephanou, 1995; Micale, et al., 1996; Hernandez et al., 2003), larvae rearing and skeletal anomalies (Divanach and Kentouri, 1982; Frenicevic, 1989; Marangos, 1995; Favaloro et al., 2002; Boglione et al., 2003a), morphology (Sara et al., 1999; Loy et al., 2000; Favaloro and Mazzola, 2000, 2003; Palma and Andrade, 2002), and nutrition and growth (Gatland, 1995; Katavic et al., 2000; Hernandez et al., 2001; Atienza et al., 2004). However, lit-

* Corresponding author. Tel: +90-232-3434000/5221, fax: + 90-232-4636050, e-mail: kfirat@sufak.ege.edu.tr

erature about its early life history is scarce or limited to embryonic and larvae development in captivity (Kentouri, 1983; Faranda, 1985; Boglione et al., 2003b).

Water temperature during spawning and egg incubation is particularly important in determining egg quality. Temperature can affect the metabolism, activity, and structure of developing embryos (Kinne and Kinne, 1961). The relationship between incubation temperature and embryonic development of sharpsnout seabream has yet to be described. The aim of this study was to provide information about the effects of temperature on the length of the incubation period and morphogenesis of sharpsnout seabream eggs.

Materials and Methods

Twenty male $(0.9\pm0.1 \text{ kg} \text{ mean wt})$ and 20 female $(1.2\pm0.2 \text{ kg} \text{ mean body wt})$ sharpsnout seabream broodstock were selected from wild breeders and stocked in a 10-m³ tank with a seawater flow of 1.5 m³ per h. The fish were hand-fed once a day to satiation at noon with approximately equal amounts (by weight) of a moist pelleted broodfish diet, cuttlefish (Sepia officinalis), and shrimp (Palaemon elegans).

The experiment began after observing the first batch of eggs. Eggs laid in the broodstock tank flowed into a recouper via a guiding panel in the tank. A sufficient amount of eggs was collected in about half an hour. The eggs were immediately taken out of the recouper, and floating dead eggs were separated from live eggs. The diameters of 100 eggs were measured to the nearest 0.025 mm using an ocular micrometer. The water temperature at spawning was 24°C.

Approximately 2000 fertilized eggs were placed in each incubator (1.5-I glass beakers). Thirty-three incubators were used for triplicates of each temperature treatment (12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32°C). The eggs were allowed to gradually adapted to the test temperature in 15 min. The adaptation period was limited to 15 min to be able to observe egg development at the test temperature from the very first cleavage. To control temperature, glass beakers were placed in 75 x 40 x 25 cm aquaria filled with water that was electrically heated or cooled by adding water from a cooling system. The water in the aquaria circulated by aeration supplied at 35 ml/min. Temperatures in the glass beakers were measured hourly with a calibrated thermometer to $\pm 0.05^{\circ}$ C. The water flow was adjusted to exchange 15% of the total water volume every hour. Dead eggs were removed immediately.

At least 15 eggs were removed for examination from each beaker every 15 min during the first 13 hours, and every hour thereafter (as in Jennings and Pawson, 1991), resulting in examination of 45 eggs from each temperature treatment at each sampling. Egg development was described prior to fixation in 4% distilled water formaldehyde buffered to pH 7 with sodium acetate. Eggs incubated at 24°C were photographed at one-hour intervals throughout development. The experiment terminated when 50% of the viable eggs at each temperature had hatched, or when all eggs were dead.

Development was divided into stages using descriptions of fresh eggs, examinations of preserved eggs, and the photographic record (Nikon coolpix 5 Mb). Criteria for determining stages was according to Jennings and Pawson (1991): (a) stage descriptions must be based on unambiguously defined and clearly identifiable embryonic events; (b) stages must begin and end concurrently in fresh and preserved eggs of the same age; (c) stages must be sufficiently different to allow precise identification of ageing; (d) transition between stages must be shorter than stage durations. We selected seven stages to describe sharpsnout seabream egg development from fertilization to hatching (Table 1; Fig. 1). The first stage was subdivided into six substages (Table 2) based on the number of cells, as suggested by Simpson (1971), Nichols (1989) for plaice (Pleuronectes platessa), and Jennings and Pawson (1991) for sea bass (Dicentrarchus labrax). The time required to reach the midpoint of each stage was calculated at each temperature as the mean egg age during the transitional periods before and after each stage. When consecutive samples conTable 1. Development stages of sharpsnout seabream eggs (see Fig. 1).

Stage Description

- From fertilization until 7th division into 128 cells (in fresh material) or until individual cells can no longer be distinguished within the blastodisc (in preserved material).
- 2 Begins with completion of the 7th division (fresh material) or when individual cells can no longer be distinguished within the blastodisc (in preserved material). Ends when blastodisc margin begins to thicken and germ ('signet') ring appears.
- 3 From first appearance of germ ring until half the yolk mass is enveloped.
- 4 Begins with germ ring enveloping half yolk mass; ends with blastopore closure.
- 5 From blastopore closure until embryo extends around 2/3 of the internal circumference of the egg.
- 6 Begins with embryo extending around 2/3 of the internal circumference of the egg; ends when it extends around 3/4.
- 7 From embryo extending around 3/4 of the internal circumference of the egg, until hatching.

tained eggs in different stages but the transitional period was not observed, the time of transition was estimated as the mean of the two sampling times.

Since adaptation of the eggs to the test temperatures was limited to 15 min, temperature shocks may have influenced the survival rates of the different treatments in different manners. Therefore, egg survival was not a concern in this study.

At the end of experiment, regression analysis of development time per mean incu-

bation temperature was carried out for each group. As samples were counted and all stage changes were determined in all samples, there was no need for statistical analysis.

Results

Temperatures were maintained within 0.5°C of the mean for each treatment. Oxygen saturation was over 85%, salinity was 37.5‰, and pH around 7.8. Ammonia and nitrite were always <0.01 mg/l.

The sharpsnout seabream eggs were buoyant, transparent, and typical of sparid eggs. They ranged in diameter from 0.807 to 0.832 mm with a mean of 0.816±0.008 mm and contained a single unpigmented oil globule (0.250±0.001 mm diameter).

Embryonic development was completed in treatments ranging 16-28°C but cells did not divide at 12 and 32°C (Table 3). Total mortality was observed at stage 2 at 14°C, and eggs at 30°C died in stage 6. Rate of development was inversely related to temperature (Figs. 2,3).

The relationship between temperature and development stage was expressed by the relationship $\ln_{age} = a + b$ (temperature), where a and b are coefficients calculated by linear regression analysis (Table 4). The coefficients of determination, r, indicate that the regression analysis provided a reliable formula for predicting the relationship between temperature and age for the seven main stages and six subdivisions of stage 1. The minimum precision of ageing from stage 1 to stage 7, based on the maximum age differences between consecutive stages at the lowest successful incubation temperature, was approximately 7 h and 10 min (Table 1) whereas this was ±0:35 h in the subdivisions of stage 1 (Table 2).

Discussion

Broodstock spawning, embryonic development, larvae survival, and growth occur within a narrow range of water temperatures. Since incubation temperature directly affects the timing of embryonic development, it also determines the hatching rate. Incubation temperatures strongly affect metabolism, activity,



Fig. 1. Stages of embryonic development in sharpsnout seabream eggs at 24°C (see Table 1).

and structure of the developing embryo and, therefore, are particularly important in affecting egg quality (Kinne and Kinne, 1961; Blaxter, 1969; Claireaux and Lagardere, 1999; Conides and Glamuzina, 2001). Generally, lower temperatures retard the rate of embryonic development of fish and higher temperatures accelerate it. As for other species, the embryonic development of *D. puntazzo* was likewise influenced by water temperature.

Each developmental life stage requires an optimal temperature that varies among species. The optimal temperature range for gilthead sea

bream (*Sparus aurata*) is fairly wide, 19±3°C (Polo et al., 1991). For European sea bass (*Dicentrarchus labrax*), the optimal range is 15-17°C (Conides and Glamuzina, 2001). For red sea bream (*Pagrus major*) and common dentex (*Dentex dentex*), eggs successfully hatched in 14.5-25.6°C and 12-18°C, respectively (Mihelakakis and Yoshimatsu, 1998; Saka et al., 2004). Sole (*Solea solea*) most successfully hatched at 8-12°C (Baynes et al., 1993). In the present experiment, the optimum temperature was 24°C, with lower and upper limits of 14°C and 28°C.

It is frequently difficult to maintain labora-

108

Table 2. Early development substages of sharpsnout seabream eggs. Stage starts and ends when a complete membrane through the dividing cytoplasm is formed.

Stage	Description
1a	From the undivided egg cytoplasm to 2 cells
1b	From 2 to 4 cells
1c	From 4 to 8 cells
1d	From 8 to 16 cells
1e	From 16 to 32 cells
1f	From 32 to 64 cells

tory broodstock and obtain naturally fertilized eggs within minutes of release. Stripping wild fish is often the only option (Jennings and Powson, 1991) and artificial fertilization is often used to obtain eggs for developmental studies (Riley, 1974; Thomson and Riley, 1981). The eggs in this study were obtained and transferred to experimental beakers immediately after fertilization. In this way, development of the eggs in the broodstock tank at the temperature of 24°C was prevented. Further, eggs were adapted to the experimental temperature within only 15 min to assure that the experimental temperature affected development in the earliest stages.

The morphology of the eggs in this study was similar to that found by Faranda et al. (1985). Cleavage did not occur below 14°C, and cell division was absent, highly asynchronous, or produced irregularly sized cells above 30°C, indicating that temperatures beyond 22±6°C may drastically reduce egg survival. Total mortality was observed before egg development was complete at temperatures of 12, 14, 30 and 32°C.

Mihelakakis and Yoshimatsu (1998)

reported that if there is more than a 2°C difference between the spawning and incubation temperatures, at least one hour is needed for each degree of change to determine correct survival rates. In this study, the eggs were rapidly adapted to the experimental temperature and thermal shocks may have affected survival. Therefore, hatching and deformity rates were not studied.

In conclusion, there is a strong relationship between temperature and embryonic development of D. puntazzo eggs. Water temperature should be maintained with minimal fluctuation, preferably no more than ±1°C from the optimal level (Watson and Chapman, 2002). By maintaining the incubation temperature within the proper level, normal embryonic development occurs, many hatched larvae can be produced, and mass larviculture of this species can be developed. Temperature fluctuations or unsuitable temperatures can cause poor embryonic survival, low hatching and growth rates, deformities, and increased disease. The survival and deformation rates in eggs of this species at various temperatures remain to be investigated. Further investigations of the effects of other biotic and abiotic factors on the embryology of this highly commercial species will advance its aquaculture.

Acknowledgements

We thank the staff of the Teknomar Sea Fish Broodstock Center where the experiments were conducted (Akuvatur Marine Product Inc., Izmir, Turkey) for their technical and financial assistance. Our thanks are also extended to Dr. Huseyin Ozbilgin for his comments on the text.

References

Atienza M.T., Chatzifotis S. and P. Divanach, 2004. Macronutrient selection by sharp snout seabream (*Diplodus puntazzo*). Aquaculture, 232:481-491.

Baynes S.M., Howell B.R. and T.W. Beard, 1993. A review of egg production by captive sole Solea solea (L). Aquacult. Fish. Management, 24:171-180.

Blaxter J.H.S., 1969. Development: eggs and larvae. pp. 177-252. In: W.S. Hoar, D.J.

Treatment	Replicate	Length of experiment (h:min)	Temperature (°C)			
			Min	Max	Mean	SD
12°C	1	*	11.7	12.3	12.09	0.202
	2		11.8	12.1	12.11	0.156
	3		11.8	12.2	12.08	0.117
14ºC	1	15:10*	13.5	14.1	14.02	0.121
	2		13.8	14.3	14.07	0.132
	3		13.8	14.3	14.03	0.109
16ºC	1	56:10	15.4	16.2	16.09	0.186
	2		15.9	16.1	16.02	0.211
	3		15.6	16.3	16.02	0.174
18ºC	1	47:20	17.6	18.1	17.92	0.202
	2		17.8	18.2	18.01	0.185
3	3		17.7	18.6	18.02	0.131
20°C	1	33:00	19.8	20.0	19.94	0.121
	2		19.7	20.1	19.96	0.102
	3		19.8	20.1	19.92	0.032
22ºC	1	28:50	21.9	22.1	21.96	0.122
	2		22.1	22.0	22.05	0.098
	3		22.2	22.0	21.56	0.207
24ºC	1	26:20	23.4	24.2	23.81	0.106
	2		23.6	24.3	23.78	0.127
	3		23.7	24.2	23.62	0.154
26ºC	1	22:00	25.9	26.2	26.09	0.118
	2		25.8.	26.1	26.01	0.111
	3		25.9	26.1	26.0	0.141
28ºC	1	20:00	27.6	28.2	28.11	0.107
2	2		27.8	28.1	27.95	0.241
	3		27.9	28.3	28.18	0.227
30°C	1	11:00*	29.6	30.1	30.02	0.207
	2		29.9	30.2	30.11	0.178
	3		29.8	30.1	29.95	0.189
32ºC	1		31.9	32.1	32.08	0.174
	2		31.8	32.0	31.94	0.312
	3		31.9	32.1	32.02	0.201

Table 3. Temperatures during incubation of sharpsnout seabream eggs.

* total mortality

110



Fig. 2. Development of sharpsnout seabream eggs in relation to temperature during Stage 1.



Fig. 3. Development of sharpsnout seabream eggs in relation to temperature during Stages 1-7.

Table 4. Regression coefficients for the relationship between the natural logarithm of sharpsnout seabream egg ages (in hours) and temperature (°C) at developmental stage midpoints, according to the equation $ln_{age} = a + b$ (temperature).

Stage	а	b	r
1a	1.4320	-1.0803	0.98
1b	1.5918	-1.0468	0.92
1c	2.8153	-1.1737	0.91
1d	3.2095	-1.1310	0.98
1e	6.8182	-1.3175	0.98
1f	9.5850	-1.3703	0.98
1	576.67	-1.6302	0.95
2	2269.4	-1.7968	0.98
3	2757.5	-1.7576	0.98
4	112160	-2.1245	0.98
5	9847.5	-2.0107	0.98
6	8059.9	-1.8697	0.97
7	8619.2	-1.8307	0.98

Randall (eds.). *Fish Physiology*, Vol. 3. Academic Press, New York.

Boglione C., Costa C., Di Dato P., Ferzini G., Scardi M. and S. Cataudella, 2003a. Skeletal quality assessment of reared and wild sharpsnout sea bream and pandora juveniles. *Aquaculture*, 227:373-394.

Boglione C., Giganti M., Selmo C. and S. Cataudella, 2003b. Morphoecology in larval fin-fish: a new candidate species for aquaculture, *Diplodus puntazzo* (Sparidae) *Aquacult. Int.*, 11:17-41.

Claireaux G. and J.P. Lagardere, 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J. Sea Res.*, 42:157-168.

Conides A. and B. Glamuzina, 2001. Study on the effects of rearing density, temperature and salinity on hatching performance of the European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquacult. Int.*, 9:217-224. **Divanach P. and M. Kentouri**, 1982. Utilisation des techniques extensive pour la production a grand échelle d'alevins du ar *Puntazzo puntazzo* (Poisson, Teleost, Sparidae). *Comp. Rend. Acad. Sci. Park*, 294(111):1017-1019.

Faranda F., Cavaliere A., Lo Paro G., Manganaro A. and A. Mazzola, 1985. Preliminary studies on reproduction of *Puntazzo puntazzo* (Gmelin, 1789) under controlled conditions. *Aquaculture*, 49:111-123.

Favaloro E. and A. Mazzola, 2000. Meristic character analysis and skeletal anomalies during growth in reared sharpsnout seabream. *Aquacult. Int.*, 8:417-430.

Favaloro E., Lopiano I. and A. Mazzola, 2002. Rearing of sharpsnout sea bream (*Diplodus puntazzo*, Cetti 1777) in a Mediterranean fish farm: monoculture versus polyculture. *Aquacult. Res.*, 33:137-140.

Favaloro E. and A. Mazzola, 2003. Shape

change during the growth of sharpsnout seabream reared under different conditions in a fish farm of the southern Tyrrhenian Sea. *Aquacult. Eng.*, 29:57-63.

Frenicevic V., 1989. Preliminary results on larviculture of *Puntazzo puntazzo* (Gmelin 1789) (Pisces, Sparidae). In: N. De Pauw, E. Jaspers, H. Ackefors, N. Wilkins (eds.). *Aquaculture - A Biotechnology in Progress*. Eur. Aquacult. Soc., Bredene, Belgium.

Gatland P., 1995. Growth of *Puntazzo puntazzo* in cages in Selonda Bay, Corinthos, Greece. pp. 51-55. In: *Marine Aquaculture Finfish Species*. Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), June 14-17, Nicosia, Cyprus. CIHEAM-IAMZ, Zaragoza.

Georgiou G. and D. Stephanou, 1995. Contribution to the study of maturation and spawning problems of the sharpsnout seabream (*Puntazzo puntazzo*). pp. 47-50. In: *Marine Aquaculture Finfish Species*. Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), June 14-17, Nicosia, Cyprus. CIHEAM-IAMZ, Zaragoza.

Hernandez M.D., Egea M.A., Rueda F.M., Aguado F., Martinez F.J. and B. Garcia, 2001. Effects of commercial diets with different P/E ratios on sharpsnout seabream *Diplodus puntazzo* growth and nutrient utilization. *Aquaculture*, 195:321-329.

Hernandez M.D., Egea M.A., Rueda F.M., Martinez F.J. and B. Garcia, 2003. Seasonal condition and body composition changes in sharpsnout seabream (*Diplodus puntazzo*) raised in captivity. *Aquaculture*, 220:569-580. Jennings S. and M.G. Pawson, 1991. The development of bass eggs in relation to temperature. *J. Marine Biol. Assoc. UK*, 71:107-116.

Katavic I., Grubisic L. and N. Skakelja, 2000. Growth performance of pink dentex as compared to four other sparids reared in marine cages in Croatia. *Aquacult. Int.*, 8: 455-461.

Kentouri M. and P. Divanach, 1983. Sur l'utilisation des critères comportementaux pour déterminer l'état de santé et l'évolution probable des élevages de poissons marins. 1. Cas des prélarves et des larves de *Diplodus sargus, Sparus aurata, Puntazzo puntazzo, Lighognathus mormyrus. Bases Biologiques de l'Aquaculture,* 1:525-538.

Kinne O. and E.M. Kinne, 1961. Rates of development in embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen combinations. *Can. J. Zool.*, 40:231-253. Loy A., Busilacchi S., Costa C., Ferlin L. and S. Cataudella, 2000. Comparing geometric morphometrics and outline fitting methods to monitor fish shape variability of *Diplodus puntazzo* (Teleostea: Sparidae). *Aquacult. Eng.*, 21:271-283.

Marangos C., 1995. Larviculture of the sheepshead bream, *Puntazzo puntazzo* (Gmelin 1789) (Pisces Sparidae). pp. 41-46. In: *Marine Aquaculture Finfish Species*. Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), June 14-17, Nicosia, Cyprus. CIHEAM-IAMZ, Zaragoza.

Micale V., Perdichizzi F. and G. Basciano, 1996. Aspects of the reproductive biology of the sharpsnout seabream *Diplodus puntazzo* (Cetti, 1777). 1. Gametogenesis and gonadal cycle in captivity during the third year of life. *Aquaculture*, 140(3):281-291.

Mihelakakis A. and T. Yoshimatsu, 1998. Effects of salinity and temperature on incubation period, hatching rate and morphogenesis of the red sea bream. *Aquacult. Int.,* 6:171-177.

Nichols J.H., 1989. The diurnal rhythm in spawning of plaice (*Pleuronectes platessa*) in the southern North Sea. *J. Conseil*, 45:277-283.

Riley J.D., 1974. The distribution and mortality of sole eggs in shore areas. pp. 39-52. In: J.H.S. Blaxter (ed.). *The Early Life History of Fish*. Springer-Verlag, Berlin.

Palma J. and J.P. Andrade, 2002. Morphological study of *Diplodus sargus, Diplodus puntazzo,* and *Lithognathus mormyrus* (Sparidae) in the eastern Atlantic and Mediterranean Sea. *Fish. Res.,* 57:1-8.

Polo A., Yufera M. and E. Pascual, 1991. Effects of temperature on egg and larval development of *Sparus aurata* L. *Eur. Aquacult. Soc. Spec. Publ.* 10:207-208.

Sara M., Favaloro E. and A. Mazzola, 1999. Comparative morphometrics of sharpsnout seabream (*Diplodus puntazzo* Cetti, 1777), reared in different conditions. *Aquacult. Eng.*, 19:195-209.

Saka S., Firat K. and D. Coban, 2004. The development of the common dentex (*Dentex dentex*) eggs in relation to temperature. *Aquacult. Res.*, 35:224-231.

Simpson A.C., 1971. Diel spawning behaviour in populations of plaice, dap, spart and pilchard. *J. Conseil*, 34:58-64.

Thompson B.M. and J.D. Riley, 1981. Egg and larval development studies in the North Sea cod (*Gadus morhua*, L.). Rapport et Proces-verbaux des Reunions. *Conseil Int. pour l'Exploration de la Mer*, 178:553-559. Tortonese E. 1970. *Osteichtyes (Pesci: Ossei)*, Part I. Edizioni Calderini, Bologna. Watson C.A. and A. Chapman, 2002. Artificial Incubation of Fish Eggs. Dept. Fish. Aquat. Sci., Inst. Food Agric. Sci., Univ. Florida, fact sheet FA-32.