J. Fish Biol. (1987) **31**, 697–704

Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*

D. A. BENGTSON*†, R. C. BARKMAN‡ AND W. J. BERRY†§

**Department of Food Science & Technology, Nutrition & Dietetics, University of Rhode Island, Kingston, Rhode Island 02881, U.S.A., ‡Department of Biology, Springfield College, Springfield, Massachusetts 01109, U.S.A. and §Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881, U.S.A.*

(*Received 11 June 1986, Accepted 17 March 1987*)

Eggs were stripped from gravid Atlantic silversides collected on two occasions, once during the early part and once during the late part of the natural spawning season. Unfertilized egg diameter was not correlated with length of the female, nor was it significantly larger during the early part of the season. Eggs were fertilized and incubated in the laboratory. Larval length at hatch was measured every 24 h during the hatching period after embryos were incubated at 18 or 25° C. Lower incubation temperature caused a significantly greater length at hatch for the offspring of each of the 20 females studies. In most cases (17 out of 20 at 25° C, 10 out of 20 at 18° C), there was a significant decrease in length at hatch during the hatching period for a given female's eggs incubated at a given temperature. In the natural environment, larvae hatched early in the season under cooler temperatures could average 12% longer than those hatched later under warmer temperatures, and therefore may have a greater chance of survival. The results help to explain the observation that field-caught *M. menidia* that hatched early in the season are larger at any given age than those that hatched late in the season.

I. INTRODUCTION

The Atlantic silverside, *Menidia menidia*, is an estuarine member of the family Atherinidae that occurs along the east coast of North America from the Gulf of St. Lawrence to north-eastern Florida. It occupies shallow estuaries during spring, summer and autumn (Bengtson, 1982; Conover & Ross, 1982) but moves to deeper water in winter (Conover & Murawski, 1982). Spawning generally occurs on a semilunar cycle (Middaugh, 1981; Conover & Kynard, 1984) during spring and early summer throughout the species' range. In Rhode Island, U.S.A. waters specifically, spawning occurs from early May to early July (Bengtson, 1982).

Although the species has little commercial value, it is important in estuarine trophodynamics as a zooplankton consumer and as food for commercial species. Because of these factors and the ease with which it can be cultured in the laboratory, it is used by the U.S. Environmental Protection Agency in toxicity testing. It is particularly valuable for early life-stage toxicity tests (Goodman *et al.*, 1985) in which fertilized embryos are reared for 28 days, through hatching and early larval development, in a series of toxicant concentrations. Survival is observed during the test, and growth of the fish is measured at the end of the experiment.

†Address correspondence to: U.S. Environmental Protection Agency, South Ferry Road, Narragansett, Rhode Island 02882, U.S.A.

0022-1112/87/011697+08 $03.00/0 © 1987 The Fisheries Society of the British Isles

Conover & Kynard (1981) reported that temperature can affect the sex determination of *M. menidia*, probably around the time of metamorphosis. They showed that cooler temperatures cause a preponderance of females to be produced, whereas warmer temperatures cause production of males, and they speculated that this phenomenon accounts for the larger size of females, compared to males, that is observed later in the summer. Barkman *et al.* (1981) noted that silversides that hatch early in the season are longer at any given age (in days) than are those that hatch late in the season. Their data suggested that the size difference is probably based on events occurring at hatch or during early larval stages. Thus, several questions arise with regard to temperature, time of spawning season, and size of *M. menidia*. However, no data are available on the variability in size of eggs during the spawning season and among females, or in length at hatch.

This study was conducted to determine (a) whether maternal size or time of spawning season affects the size of eggs and newly-hatched larvae, as shown for other species by Gall (1974), Ware (1977) and Kazakov (1981), and (b) whether length at hatch is affected by temperature, as shown for other species by Alderdice & Velsen (1971), Alderdice & Forrester (1974) and Westernhagen (1983).

II. METHODS

Field collections of adult *M. menidia* were made in two Rhode Island, U.S.A. estuaries, using a 30-m beach seine with 6·3-mm mesh. The first collection (early season, ES) was made in the Pettaquamscutt River estuary on 19 May 1983, and the second (late season, LS) at the mouth of Bissell Cove on Narragansett Bay on 23 June 1983. Collection dates were chosen to coincide with spawning periods associated with the full moon, and the different collection sites were needed because *M. menidia* spawns in different areas of estuaries as the spawning season progresses (Bengtson, 1982). The fish were transported live to the laboratory and used within 3 h of capture.

The basic experimental design was that fertilized eggs from a female were reared at two temperatures, 18 and 25° C, and lengths of larvae at hatch were measured. On each collection date, 10 females were selected for the experiments to represent the widest possible range of sizes of gravid females, and their standard lengths were measured to the nearest millimetre. Each female's eggs were stripped onto a 1-mm mesh nylon screen, to which eggs ready for fertilization attached by means of filamentous threads (Barkman & Beck, 1976). Previous experience has shown that eggs with filaments are ready for fertilization, while those without filaments are not. The screens were placed in filtered sea water and a small section of each screen with its attached eggs was removed so that the diameters of 10 unfertilized eggs from each female could be measured. All phases of the experiment were conducted at 10‰ salinity and all egg and larval measurements were made with a filar micrometer to the nearest 0·01 mm. Milt from a combined sample of 20 males was then poured over the eggs and 30 min was allowed for fertilization to occur. Each screen was then cut in half and each half was suspended in a 250-ml beaker containing 200 ml of sea water. One beaker was incubated at $25 \pm 1°$ C, the other at $18 \pm 1°$ C. All incubation was under a 12L:12D photoperiod. Each screen was removed from its beaker daily and placed in a new beaker. Larvae that had hatched in a beaker during the previous 24-h period were anaesthetized with MS-222, counted, and up to 20 larvae per beaker per day (if available) were measured (total length). Data presented on a daily basis are from measurements of at least 10 larvae per beaker per day.

III. RESULTS

The LS gravid females collected for this study were shorter than the ES gravid females, but produced slightly larger eggs (Table I); however, in neither case were the differences significant. Many larger LS females were collected, but they were

TABLE I. Standard lengths (S.L.) and egg diameters of 10 female *Menidia menidia* collected early (ES) and late (LS) in the spawning season and total length at hatch of their offspring incubated at two temperatures. Data are presented as mean ± 1 S.D.

	Female S.L. (mm)	Unfertilized egg diameter (mm)	Total length at hatch (mm)	
			18° C	25° C
ES	82	1·10±0·03	5·78±0·19	5·18±0·27
	85	1·12±0·04	5·88±0·19	5·36±0·24
	93	1·09±0·03	5·59±0·23	5·21±0·25
	96	1·08±0·03	5·67±0·15	5·34±0·22
	99	1·08±0·04	5·88±0·20	5·29±0·23
	99	1·09±0·05	5·74±0·24	5·12±0·33
	112	1·11±0·06	5·79±0·17	5·23±0·44
	113	1·08±0·05	5·59±0·24	5·21±0·24
	119	1·18±0·03	5·85±0·31	5·33±0·30
	130	1·06±0·05	5·59±0·16	4·83±0·25
ES grand mean	103±15	1·11±0·05	5·73±0·24	5·20±0·32
LS	83	1·09±0·06	5·68±0·13	5·21±0·29
	89	1·11±0·04	5·40±0·21	4·83±0·29
	90	1·09±0·07	5·72±0·23	5·06±0·28
	90	1·14±0·06	5·87±0·29	5·40±0·36
	95	1·09±0·03	5·53±0·19	5·32±0·35
	99	1·14±0·06	5·51±0·25	5·21±0·43
	100	1·10±0·03	5·24±0·28	4·68±0·29
	102	1·11±0·05	5·46±0·19	5·06±0·26
	104	1·11±0·03	5·45±0·24	4·99±0·38
	104	1·17±0·02	5·30±0·35	4·94±0·31
LS grand mean	96±7	1·13±0·05	5·48±0·30	5·06±0·39

not carrying fertilizable eggs. The variability in egg diameters was small, generally 5% or less, both for a given female and for all eggs measured on each collection date.

Embryos that were incubated at 25° C hatched 6–10 days after fertilization, with >75% of the hatching occurring on days 7–8, for both ES and LS groups. Embryos incubated at 18° C hatched 12–21 days after fertilization, with >75% of the hatching occurring on days 12–15, for both groups. Although actual hatching percentage was not measured, we estimate that >90% of all fertilized embryos hatched.

The newly-hatched larvae of every female tested were significantly longer when incubated at 18° C than when incubated at 25° C (Table I). The ES group averaged 0·53 mm longer (difference in grand means) at 18° C and the LS group 0·42 mm longer. Also, the larvae from the ES group at each temperature were significantly longer than the larvae from the LS group at that temperature. A ranking of the mean larval lengths by group shows that ES-18 > LS-18 > ES-25 > LS-25, and all means were significantly different ($P < 0{\cdot}05$; ANOVA with Student–Newman–Keuls *a posteriori* test). It should be pointed out that the significance of the differences may have been enhanced by the large sample sizes (a total of 3423 larvae were measured). The range in total lengths was from 3·62 to 6·57 mm.

TABLE II. Average daily total length (mm) at hatch for larvae of 10 *Menidia menidia* females collected early (ES) and late (LS) in the spawning season and incubated at 25° C. Data are means ± 1 S.D. and are based on measurements of at least 10 larvae per female per day. The larvae of fish marked by an asterisk were significantly longer on the first day of hatch than on the last

	Female S.L. (mm)	Time (days post-fertilization)			
		6	7	8	9
ES	82	—	5·23±0·22	4·94±0·31	5·19±0·25
	85*	5·41±0·17	5·39±0·23	5·18±0·27	—
	93*	5·43±0·26	5·23±0·22	5·16±0·27	4·94±0·19
	96*	5·45±0·14	5·38±0·17	5·19±0·26	5·05±0·20
	99	—	5·32±0·19	5·25±0·21	5·21±0·24
	99*	—	5·31±0·29	5·19±0·20	5·04±0·27†
	112*	—	5·42±0·25	4·98±0·46	4·97±0·42
	113*	5·42±0·14	5·22±0·24	5·15±0·20	5·06±0·27
	119*	—	5·48±0·22	5·24±0·23	5·01±0·24
	130*	5·20±0·11	4·89±0·18	4·68±0·25	4·62±0·15
LS	83*	5·39±0·20	5·17±0·17	—	
	89*	—	4·88±0·25	4·74±0·19	—
	90	—	5·00±0·26	5·08±0·30	—
	90*	5·83±0·16	5·45±0·27	5·24±0·26	—
	95*	5·49±0·18	5·33±0·32	5·15±0·45	—
	99*	5·52±0·36	5·21±0·37	4·93±0·30	—
	100*	—	4·73±0·22	4·56±0·25	—
	102*	5·23±0·18	5·02±0·26	4·97±0·25	—
	104*	5·39±0·22	4·90±0·36	4·96±0·35	4·89±0·38
	104*	5·21±0·14	4·91±0·23	4·85±0·38	4·76±0·22

†This fish also had > 10 larvae hatch on day 10, for which the average length was 4·85±0·37 mm.

Attempts at regressing egg diameter with maternal size and with length at hatch at both temperatures yielded no significant relationships ($P > 0{\cdot}05$: ANOVA for the regression). Thus, unfertilized egg diameter was unrelated to maternal size, and length at hatch was unrelated to egg diameter.

The data in Tables II and III show that there was a general tendency for length at hatch to decline as time to hatch increased, for embryos of a given female. For 27 out of 40 females tested (17 out of 20 at 25° C, 10 out of 20 at 18° C) average length at hatch was significantly greater on the first day on which > 10 larvae hatched than it was on the last day on which > 10 larvae hatched. This tendency was more common for embryos of larger females at 18° C than for embryos of smaller females at 18° C.

IV. DISCUSSION

Our finding that egg diameter is unrelated to maternal standard length was surprising because Gall (1974) and Kazakov (1981), among others, reported that relationships exist between maternal size and egg size for salmonid species.

TABLE III. Average daily total length (mm) at hatch for larvae of 10 *Menidia menidia* females collected early (ES) and late (LS) in the spawning season and incubated at 18° C. Data are means ± 1 S.D. and are based on measurements of at least 10 larvae per female per day. The larvae of fish marked by an asterisk were significantly longer on the first day of hatch than on the last

	Female S.L. (mm)	Time (days post-fertilization)					
		12	13	14	15	16	17
ES	82	—	5·88 ± 0·18	5·75 ± 0·17	5·78 ± 0·16	—	—
	85	—	—	5·94 ± 0·19	5·93 ± 0·14	—	—
	93	—	5·54 ± 0·22	5·79 ± 0·15	5·73 ± 0·14	—	—
	96	—	5·68 ± 0·10	5·74 ± 0·15	5·69 ± 0·17	5·60 ± 0·16	—
	99	—	5·85 ± 0·14	5·97 ± 0·17	5·89 ± 0·21	—	—
	99*	—	5·91 ± 0·22	5·78 ± 0·22	5·78 ± 0·26	5·62 ± 0·15	5·70 ± 0·21
	112*	—	5·85 ± 0·17	5·80 ± 0·16	5·81 ± 0·18	5·73 ± 0·13	—
	113*	—	5·87 ± 0·23	5·63 ± 0·18	5·68 ± 0·20	5·53 ± 0·17	5·56 ± 0·17†
	119*	—	6·04 ± 0·22	6·05 ± 0·20	5·97 ± 0·18	5·52 ± 0·26	—
	130	5·56 ± 0·15	5·62 ± 0·18	5·64 ± 0·12	—	—	—
LS	83	5·63 ± 0·11	5·74 ± 0·08	—	—	—	—
	89	5·45 ± 0·18	5·52 ± 0·18	5·37 ± 0·19	5·39 ± 0·20	—	—
	90	—	5·77 ± 0·21	5·77 ± 0·14	5·68 ± 0·31	—	—
	90*	—	5·96 ± 0·22	6·02 ± 0·17	5·66 ± 0·31	—	—
	95	—	5·52 ± 0·21	5·56 ± 0·17	5·54 ± 0·21	—	—
	99*	5·64 ± 0·15	5·72 ± 0·18	5·66 ± 0·12	5·57 ± 0·17	5·39 ± 0·27	5·35 ± 0·23‡
	100*	5·43 ± 0·15	5·38 ± 0·19	5·28 ± 0·16	5·22 ± 0·20	4·92 ± 0·30	—
	102*	—	5·53 ± 0·12	5·51 ± 0·12	5·49 ± 0·16	5·46 ± 0·22	5·40 ± 0·20
	104*	5·64 ± 0·16	5·62 ± 0·21	5·52 ± 0·21	5·40 ± 0·17	5·30 ± 0·19	5·29 ± 0·19
	104*	5·47 ± 0·16	5·51 ± 0·20	5·43 ± 0·17	5·44 ± 0·27	5·19 ± 0·22	—

†This fish also had > 10 larvae hatch on days 18–20, for which the average lengths were 5·41 ± 0·20, 5·42 ± 0·17 and 5·55 ± 0·25 mm, respectively.
‡This fish also had > 10 larvae hatch on day 18, for which the average length was 5·33 ± 0·22 mm.

However, Ware (1975) pointed out that egg size was remarkably constant for a given marine fish species in a given geographical area. Our finding that there was no significant decrease in egg diameter during the spawning season differed from the previous findings of Bagenal (1971) and Ware (1977). A possible explanation for the contradiction between our data and previous data is that *M. menidia* spawning adults are almost exclusively of one age-class (I), while the other species studied have several age classes, so that the size differences of females are greater. Also, most studies that showed a decrease in egg diameter during the spawning season used eggs collected from the plankton, so maternal size was not considered.

Previous studies on the effect of temperature on length at hatch have produced conflicting results. Length at hatch has been shown to increase with decreasing temperature (Alderdice & Velsen, 1971; Westernhagen, 1983), to decrease with decreasing temperature (Alderdice & Forrester, 1974), and to be unaffected by temperature (Blaxter & Hempel, 1963). It may be that the effects vary with species and depend also on the experimental temperatures chosen. Our study indicates that *M. menidia* falls into the category of organisms for which length at hatch increases with decreased temperature, at least over the temperature range that we used.

The average size of larvae hatching from a group of eggs on successive days has been shown to increase from day to day for some species (Alderdice & Forrester, 1974) and to first increase, then decrease, for other species (Alderdice & Velsen, 1971; Westernhagen, 1983). We found no reports in the literature of a constant decrease in length at hatch on successive days, as we found for *M. menidia*. Our results suggest that embryos (within a group) that take longer to hatch put more energy (i.e., yolk) into metabolic costs than into growth, especially at 25° C where metabolic costs may be higher. A more detailed study of yolk utilization efficiency is necessary to determine the physiological basis of this phenomenon.

The relevance of our findings for toxicologists who conduct early life-stage tests with *M. menidia* is twofold. First, we have shown that the eggs from a natural population are about 1·1 mm in diameter and vary relatively little with female size or time of spawning season. The eggs used for early life-stage tests are often obtained from spawnings of laboratory-cultured fish. Thus, researchers who wish to apply test results to field populations may wish to compare their data on egg size to our data. Second, tests conducted at 25° C, which is the temperature recommended by Goodman *et al.* (1985), will probably yield larvae that are shorter at hatch than they would be if tests were conducted at cooler temperatures. Choice of food organisms for the first-feeding larvae may then become important, because the mouth size of those shorter larvae may also be reduced.

The general ecological relevance of our data is based on the assumption that increased larval size is advantageous. Cushing (1974) has pointed out that larger size for a fish larva means that it can ingest a greater range of sizes of food particles and that it is less susceptible to predation. Atlantic silverside larvae that hatch early in the season under cool temperatures (e.g., 18° C) could be 12% longer than those that hatch late in the season under warm temperatures (e.g., 25° C) (5·73 mm *v.* 5·06 mm; Table I). A simple simulation based on known growth rates for field-captured *M. menidia* (Barkman & Bengtson, 1987) and lengths at hatch of 4·5 or 5·5 mm shows that a 1 mm difference in length at hatch translates into a size difference of approximately 12 mm for juvenile *M. menidia* after 70 days of growth

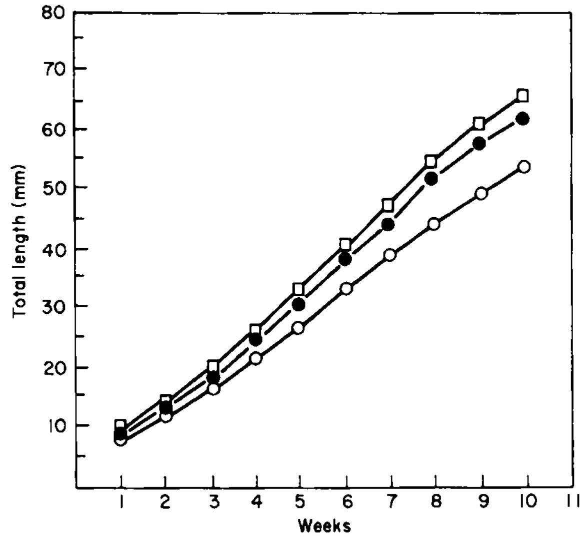

FIG. 1. Simulation of *Menidia menidia* growth (length at age) during the first 10 weeks of life, based on known rates of growth in the field (Barkman & Bengtson, 1987) and assumed initial lengths at hatch of ○, 4·5 mm; ●, 5·0 mm and □, 5·5 mm.

(Fig. 1). Similarly, a model of density-dependent growth and mortality developed for striped bass larvae by Logan (1985) predicts that a 12% reduction in length at hatch would result in a 28% reduction in population size (number of larvae) after 120 days. We have, therefore, experimental evidence that temperature affects length at hatch, and theoretical evidence that this effect is ecologically important to the population. If the ES silverside larvae become predominantly female, as Conover & Kynard (1981) suggested, then the increased length at hatch only serves to enhance the subsequent size difference between males and females. The simulation (Fig. 1) shows that an increase in length at hatch from 5·0 to 5·5 mm results in a size difference of about 4 mm after 10 weeks of growth. Four millimetres is the size difference expected between 70-day-old ES fish and 70-day-old LS fish, based on the age–growth equations calculated for the two groups by Barkman *et al.*, 1981. Thus, that size difference could be ascribed to different lengths at hatch under the different temperature conditions.

This paper is Contribution Number 2211 from the University of Rhode Island, Agricultural Experiment Station and Contribution Number 633 from the U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, Rhode Island 02881, U.S.A.

References

Alderdice, D. F. & Forrester, C. R. (1974). Early development and distribution of the flathead sole (*Hippoglossoides elassodon*). *J. Fish. Res. Bd Can.* **31,** 1899–1918.

Alderdice, D. F. & Velsen, F. P. J. (1971). Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). *J. Fish. Res. Bd Can.* **28,** 1545–1562.

Bagenal, T. B. (1971). The interrelation of the size of fish eggs, the date of spawning and the production cycle. *J. Fish Biol.* **3,** 207–219.

Barkman, R. C. & Beck, A. D. (1976). Incubating eggs of the Atlantic silverside on nylon screen. *Progve Fish-Cult.* **38,** 148–150.

Barkman, R. C. & Bengtson, D. A. (1987). The record of daily growth in otoliths of Atlantic silversides, *Menidia menidia*, from field and laboratory. *J. Fish Biol.* **31,** 683–695.

Barkman, R. C., Bengtson, D. A. & Beck, A. D. (1981). Daily growth of the juvenile fish (*Menidia menidia*) in the natural habitat compared with juveniles reared in the laboratory. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer* **178,** 324–326.

Bengtson, D. A. (1982). Resource partitioning by *Menidia menidia* (L.) and *Menidia beryllina* (Cope) in two Rhode Island estuaries. Unpubl. Ph.D. thesis, University of Rhode Island.

Blaxter, J. H. S. & Hempel, G. (1963). The influence of egg size on herring larvae (*Clupea harengus* L.). *J. Cons. perm. int. Explor. Mer* **28,** 211–240.

Conover, D. O. & Kynard, B. E. (1981). Environmental sex determination: interaction of temperature and genotype in a fish. *Science* **213,** 577–579.

Conover, D. O. & Kynard, B. E. (1984). Field and laboratory observation of spawning periodicity and behavior of a northern population of the Atlantic silverside, *Menidia menidia* (Pisces: Atherinidae). *Env. Biol. Fish.* **10,** 161–171.

Conover, D. O. & Murawski, S. A. (1982). Offshore winter migration of the Atlantic silverside, *Menidia menidia*. *Fish. Bull.* **80,** 145–150.

Conover, D. O. & Ross, M. R. (1982). Patterns in seasonal abundance, growth and biomass of the Atlantic silverside, *Menidia menidia*, in a New England estuary. *Estuaries* **5,** 275–286.

Cushing, D. H. (1974). The possible density-dependence of larval mortality and adult mortality in fishes. In *The Early Life History of Fish* (J. H. S. Blaxter, ed.), pp. 103–111. New York: Springer.

Gall, G. A. E. (1974). Influence of size of eggs and age of female on hatchability and growth in rainbow trout. *Calif. Fish Game* **60,** 26–36.

Goodman, L. R., Hansen, D. J., Middaugh, D. P., Cripe, G. M. & Moore, J. C. (1985). Methods for early life-stage toxicity tests using three atherinid fishes and results with Chlorpyrifos. In *Aquatic Toxicology and Hazard Assessment: Seventh Symposium, ASTM STP 854* (R. D. Cardwell, R. Purdy & R. S. Bahner, eds), pp. 145–154. Philadelphia: American Society for Testing and Materials.

Kazakov, R. V. (1981). The effect of the size of Atlantic salmon, *Salmo salar* L., eggs on embryos and alevins. *J. Fish Biol.* **19,** 353–360.

Logan, D. T. (1985). Environmental variation and striped bass population dynamics: a size-dependent mortality model. *Estuaries* **8,** 28–38.

Middaugh, D. P. (1981). Reproductive ecology and spawning periodicity of the Atlantic silverside, *Menidia menidia* (Pisces: Atherinidae). *Copeia* **1981,** 766–776.

Ware, D. M. (1975). Relation between egg size, growth, and natural mortality of larval fish. *J. Fish. Res. Bd Can.* **32,** 2503–2512.

Ware, D. M. (1977). Spawning time and egg size of Atlantic mackerel, *Scomber scrombrus*, in relation to the plankton. *J. Fish. Res. Bd Can.* **34,** 2308–2315.

Westernhagen, H. V. (1983). Observations on the reproductive and larval biology of *Blennius pavo* (Pisces: Teleostei). *Helgolander Wiss. Meeresunters.* **36,** 323–335.