

Estimating Daily Egg Production of European Anchovy in the Adriatic Sea: A Critical Appraisal

Paco Melià*¹, Nicola Casavola² & Marino Gatto¹

¹ Dipartimento di Elettronica e Informazione, Politecnico di Milano, via Ponzio 34/5, I-20133 Milano, Italy.

² Servizio Ittico Ambientale, Provincia di Bari, via Amendola 189, I-70126 Bari, Italy.

Keywords: Stock assessment, pelagic stocks, multiple spawning fishes, daily egg production, DEPM, biomass estimation, stage-age relationship, European anchovy.

Abstract. We discuss the critical problem of estimating daily egg production in the Daily Egg Production Method (DEPM), a widely used technique for the stock assessment of multiple spawning pelagic fishes. Data collected in 1999 for the stock of European anchovy (*Engraulis encrasicolus*) in the Southern Adriatic Sea are used to study the effects of different calibration techniques. We analyze the performances of the mortality model from which daily egg production is estimated. All these calibrations are based on the Bootstrap method. Nonlinear fitting (vs. linear regression on log-transformed data) is shown to guarantee more reliable parameter estimation. We show that specifying the function assigning age to staged eggs is crucial.

Problem

The stock assessment of exploited fish populations is of fundamental importance, as it provides the basic information required for developing sustainable management policies. The availability of reliable biomass estimates allows the monitoring of stock dynamics in populations, such as multiple spawning pelagic fishes, characterized by rapid fluctuations of their biological rates. The Daily Egg Production Method (DEPM) was developed during the 1980s by the National Marine Fisheries Service (USA) to assess the northern anchovy (*Engraulis mordax*) stock, and was then applied to other American and European species (see Palomera, 2001 for an overview of the applications to Mediterranean species). The method and its application are extensively described in Lasker (1985). According to Stauffer & Picquelle (1980), the spawning biomass B is calculated as

$$(1) \quad B = P \cdot A \cdot W / (R \cdot F \cdot S),$$

* Author to whom correspondence should be addressed: paco.melia@elet.polimi.it

where P is the so-called daily egg production (number of eggs produced per day per unit area), A is the total surveyed area, W is the average weight of mature females, R is the fraction of the population that are mature females (by weight), F is the batch fecundity (number of eggs spawned per mature female per batch), and S is the fraction of mature females spawning per day (which is a measure of spawning frequency).

The most critical step when applying the DEPM is the estimation of P and S (Palomera, 2001). Here, we focus only on estimating the daily egg production P . We analyze some critical assumptions for estimating P and use data collected in 1999 in the Southern Adriatic Sea to evaluate the sensitivity of daily egg production estimates upon different underlying models of egg development and mortality and different calibration techniques. We apply nonparametric statistics, based on the Bootstrap, to calibrate the egg mortality model required to estimate P . We can thus associate probability distributions to estimates of both P and the mortality rate.

Material and Methods

Data used in this work were collected by Servizio Ittico Ambientale, Provincia di Bari, during a survey in the Southern Adriatic Sea (August-September 1999) aimed at assessing the local stock of European anchovy (*Engraulis encrasicolus*). Eggs were sampled by means of a CalVET net with a mouth area of 0.05 m², along a grid of 5 by 20 nautical miles (nmi). The net was lowered vertically to a depth of 100 m, or to near-bottom in shallow waters. In areas where active spawning was observed or was deemed likely to occur (according to previous experience), the distance between transects was reduced to 5 nmi, so as to cover spawning areas with a 5 by 5 nmi grid (Fig. 1). Sampling was conducted in accordance to the protocols described by Smith *et al.* (1985). 122 tows were made, covering an overall area of 12,862.14 km², and 326 eggs were counted from 65 positive tows (total positive area was 5,574 km²). The temperature at a depth of 20 m, averaged over the positive area, was 20.5 °C. Eggs were staged according to Moser & Ahlstrom's (1985) 11-stage classification.

The ageing of staged eggs is the first step required to estimate the daily egg production according to Picquelle & Stauffer (1985). The basic formula for assigning age to an egg from the i -th stage at temperature t is (see Lo, 1985 for more details):

$$(2) \quad y(t, i) = \bar{y}(t, i) + k - \text{remainder of } [(\bar{y}(t, i) + st)/24],$$

where

$$(3) \quad \bar{y}(t, i) = a \exp(bt + ci) i^d$$

is the average age (in hours) of an egg from the i -th stage at temperature t , k is the time of tow and st is the peak spawning time. a , b , c and d are parameters to be estimated experimentally by monitoring egg development at different temperatures; the four parameters differ between species and possibly between populations of the same species subject to different environmental conditions. Egg ageing is usually performed by means of the Fortran program STAGEAGE (Lo, 1985), based on eqs. (2) and (3) and the estimates of a , b , c and d for *Engraulis mordax*. As the software was developed in the early 1980s, eq. (3) was pre-calculated and tabulated at discrete temperature intervals to diminish computational costs. Although STAGEAGE has been widely used for two decades, no attempts were made to establish the effect of the discretization of the stage-age function on egg ageing. Therefore, here we use both the discretized and continuous form of eq. (3) to compare the resulting estimates of the daily egg production.

We test two stage-age models, differing only in parameter values (Table 1): the original one proposed by Lo (1985) for *Engraulis mordax* and the model calibrated by Motos (1994) for *Engraulis encrasicolus* in the Gulf of Biscay. We also consider two modified versions of this latter, in which we assume different values of the peak spawning time (namely 22:00 and 23:00 GMT instead of 24:00). This choice seems reasonable because of the difference in actual time between the Gulf of Biscay and the Southern Adriatic Sea (approx. 85 min.). The reproduction of *Engraulis encrasicolus* in the Southern Adriatic Sea, in fact, occurs between 20:00 and 02:00 (Caragitsou *et al.*, 2001), so 23:00 can be assumed as the mean spawning time.

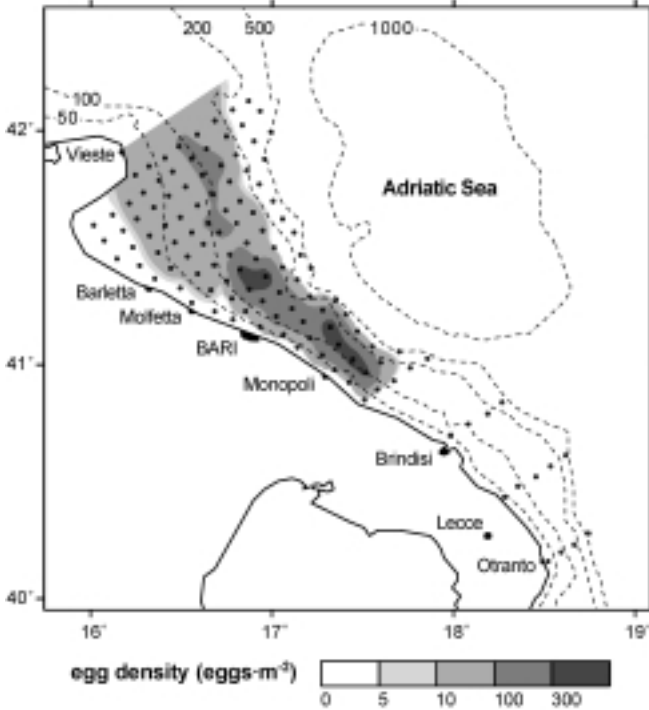


Fig. 1. The study area. Dots indicate tow stations; dashed curves identify isobaths; gray shading is proportional to measured egg density.

Aged eggs were grouped in 1/2-day categories (to diminish data variability) and used to estimate the following mortality model (modified from Picquelle & Stauffer, 1985):

$$(4) \quad P_{j1} = P_1 \cdot \exp(-Z y_j),$$

where P_{j1} is the number of eggs per unit area in the j -th age category, averaged over the total positive sampling area, P_1 is the daily egg production per unit area, Z is the rate of instantaneous egg mortality (to be estimated together with P_1), and y_j is the mean age of an egg of the j -th age class (averaged over the totality of eggs assigned to that class). Zeros that fall within the positive area (i. e., no eggs of a given age class found in a positive sample) are considered as actual data. The average daily egg production over the total surveyed area is then obtained as $P = P_1 \cdot A_1/A$, where A_1 is the positive sampling area and A the total surveyed area.

The calibration technique of the mortality model is quite important. In fact, Picquelle & Stauffer (1985) hypothesize that eq. (4) is affected by an additive error term and recommend calibrating the model by means of nonlinear fitting because a linear regression on log-transformed data could provide biased estimates. As

Table 1. Parameter sets used for the stage-age model (eq. 3).

Stock	<i>a</i> (h)	<i>b</i> (°C ⁻¹)	<i>c</i> (-)	<i>d</i> (-)	<i>st</i> (GMT h:min)
<i>E. mordax</i>	16.07	-0.1145	-0.098	1.74	22:00
<i>E. encrasicolus</i>	15.45	-0.115	-0.147	2.071	24:00*

* The original model proposed by Motos (1994) considers 24:00 GMT as the peak spawning time (*st*); here we also test *st* = 22:00, *st* = 23:00 GMT.

Table 2. Results of the model calibration by bias-corrected bootstrapping (see Efron, 1982 for details; number of Bootstrap replicates = 1000). CV is the coefficient of variation.

Stage-age model	calibration technique ^a	P_1	CV	Z	CV	expl. var. %
<i>E. mordax</i>	C-NL	72.22	0.27	0.65	0.26	73.4
<i>E. mordax</i>	C-RL	71.12	0.33	0.51	0.64	48.1
<i>E. mordax</i>	D-NL	78.05	0.25	0.77	0.22	73.8
<i>E. mordax</i>	D-RL	109.96	0.35	1.44	0.47	57.3
<i>E. encrasicolus</i> , st = 24:00	C-NL	64.27	0.28	0.56	0.34	50.6
<i>E. encrasicolus</i> , st = 24:00	C-RL	61.10	0.29	0.56	0.44	36.0
<i>E. encrasicolus</i> , st = 24:00	D-NL	66.47	0.27	0.62	0.33	53.5
<i>E. encrasicolus</i> , st = 24:00	D-RL	70.93	0.29	0.85	0.34	49.8
<i>E. encrasicolus</i> , st = 23:00	C-NL	64.04	0.28	0.56	0.33	61.6
<i>E. encrasicolus</i> , st = 23:00	C-RL	64.45	0.31	0.53	0.49	52.1
<i>E. encrasicolus</i> , st = 23:00	D-NL	65.66	0.28	0.62	0.32	62.2
<i>E. encrasicolus</i> , st = 23:00	D-RL	72.87	0.30	0.82	0.32	57.9
<i>E. encrasicolus</i> , st = 22:00	C-NL	65.11	0.26	0.56	0.30	82.1
<i>E. encrasicolus</i> , st = 22:00	C-RL	72.45	0.29	0.59	0.45	81.4
<i>E. encrasicolus</i> , st = 22:00	D-NL	66.97	0.27	0.64	0.30	77.6
<i>E. encrasicolus</i> , st = 22:00	D-RL	96.40	0.28	1.11	0.27	75.7

^aC = continuous temperature stage-age model, D = discrete temperature stage-age model; NL = nonlinear fitting on raw data, RL = linear regression on log-transformed data.

earlier stages are more affected by stochasticity than later stages (Uriarte & Motos, 1998), one can, however, assume a multiplicative error in eq. (4). This can be a valid alternative, especially if uncertainty is considered to be caused by environmental rather than demographic stochasticity (Dennis *et al.*, 1991). Under this alternative, linear regression on log-transformed data may be reasonable. We consequently test both calibrating techniques to determine their different effects on the final estimate of the daily egg production. To obtain a measure of the uncertainty associated with parameter estimates, we use the most classical nonparametric method, the Bootstrap (Efron, 1979). By resampling (with replacement) positive tows from the original data set, one obtains a Bootstrap replicate of the same size. The replicate is then used to calibrate the model again; the procedure is repeated n times, depending on the desired accuracy (here $n = 5000$), and provides an empirical probability distribution of the model parameters. Finally, a bias correction is applied to parameter estimates according to Efron (1982).

Results

The results obtained with the previously described techniques are summarized in Table 2. Estimates of the daily egg production P_1 vary from 61.10 to 109.96 eggs \cdot m⁻² \cdot d⁻¹, with most values between 64 and 78. The corresponding coefficients of variation (CV) range between 0.25 and 0.35. As for the mortality rate Z, its estimates range between 0.51 and 1.44 d⁻¹, whereas its CVs vary from 0.22 to 0.64. The best performances (in terms of explained variance) are obtained by using Motos' (1994) parameter set, modified by considering a peak spawning time at 22:00 GMT; this seems to confirm the spawning delay between Spanish and Italian anchovy populations, although a detailed analysis of adult gonads would be desirable to verify this assumption.

The use of a continuous temperature stage-age model apparently provides the best results in this case. This, however, does not hold for the suboptimal stage-age parameter

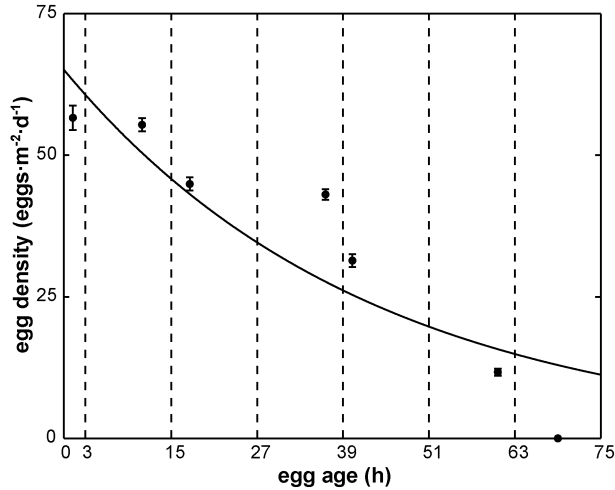


Fig. 2. Egg density per unit area per day as a function of age. Dots represent egg densities by age class (\pm standard deviation), which were used to calibrate the mortality model (eq. 4); the solid line represents the best performing curve (Motos' 1994 parameter set with the peak spawning time set at 22:00 GMT). Age classes (divided by dashed lines) have a 12-h span, except the first which has a 3-h span (newly spawned eggs; see Lo, 1985).

sets. Nonlinear fitting on raw data always provides better performances than linear regression on log-transformed data. Fig. 2 shows the best performing survivorship curve, obtained with the modified version of Motos' parameter set via nonlinear fitting and using a continuous temperature stage-age model. Note that, as suggested in Motos (1994), newly spawned eggs were considered only when sampled outside the main spawning period (in our case, after 02:00 GMT). Motos (1994) also suggests that eggs older than 90 % of the total incubation time – approximately 36 h at 20.5 °C, according to Regner's (1985) equation for estimating incubation time as a function of water temperature – should not be considered. This, however, seems to be a rather brief time compared to egg persistence in water according to our data. Therefore, we decided to include the late stages too.

Discussion

We have compared different parameter sets of the stage-age model. Motos' (1994) set for *E. encrasicolus*, suitably adjusted in the value of the peak spawning time, yields the best performances (explained variance: 82.1 %) regardless of the calibration technique. On the other hand, the model with the peak spawning time set at 24:00 GMT seems to fit the data very poorly, even worse than Lo's (1985) model, which was calibrated on *E. mordax*. This may indicate that the quality of stage-age models relies mainly on correctly identifying the actual spawning time.

Note, however, that the use of Lo's parameter set provides good results only when the mortality curve is calibrated by means of a nonlinear fitting algorithm, whereas it provides out-of-range estimates and high coefficients of variation for both P_1 and Z

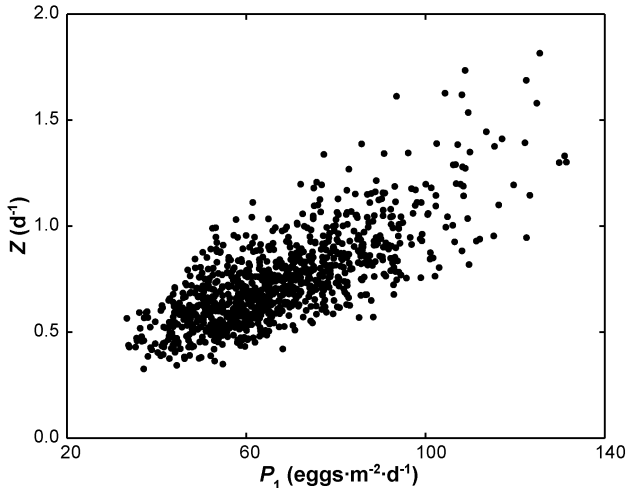


Fig. 3. Distribution of the Bootstrap replicates of P_1 and Z obtained with the stage-age model of Fig. 2.

when the calibration is performed by linear regression on log-transformed data. This would confirm that nonlinear fitting is preferable, as suggested by Picquelle & Stauffer (1985); however, the high variability in the estimates could obfuscate the differences between fitting techniques.

As for the discrete vs. continuous formulation of the stage-age model, the results do not clearly indicate which technique should preferably be applied based solely on the fitting performances. However, since the computational cost of evaluating function (3) is no longer a problem with modern computers, considering a continuous range of temperature instead of using pre-calculated tables would be conceptually more correct.

The two parameters of the mortality model are highly correlated, as clearly shown by the scatterplot in Fig. 3, where the results of the Bootstrap replication (for the best performing model of Fig. 1) are plotted in the $P_1 - Z$ plane. The correlation between P_1 and Z is possibly one of the major causes of variability in the estimates. Note also that the marginal distribution of both parameters is log-normally distributed, as shown in Fig. 4, so the logarithms of parameter values can be considered as linked by a joint bivariate normal distribution.

Coefficients of variation for P_1 and Z are comparable to those provided by previous applications to Mediterranean anchovies (see Palomera, 2001). The range of variability obtained by applying different calibration methods suggests that a rather high degree of uncertainty intrinsically affects egg production estimates.

The use of eq. (4) to estimate P_1 requires satisfying a number of critical assumptions. First, the mortality rate must not vary with egg age or with development stage, a hypothesis that is often violated due to predation on early stages (Hunter & Lo, 1997). Second, spawning must occur at a fixed time of day (Picquelle & Stauffer, 1985; Palomera, 2001). Furthermore, to ensure that the life table obtained by aggregating the data collected during the survey coincides with the dynamic life table of a cohort of eggs released at a given time, the total number of eggs spawned per day must be constant throughout the duration of the survey. This implies that the abundance of the reproduc-

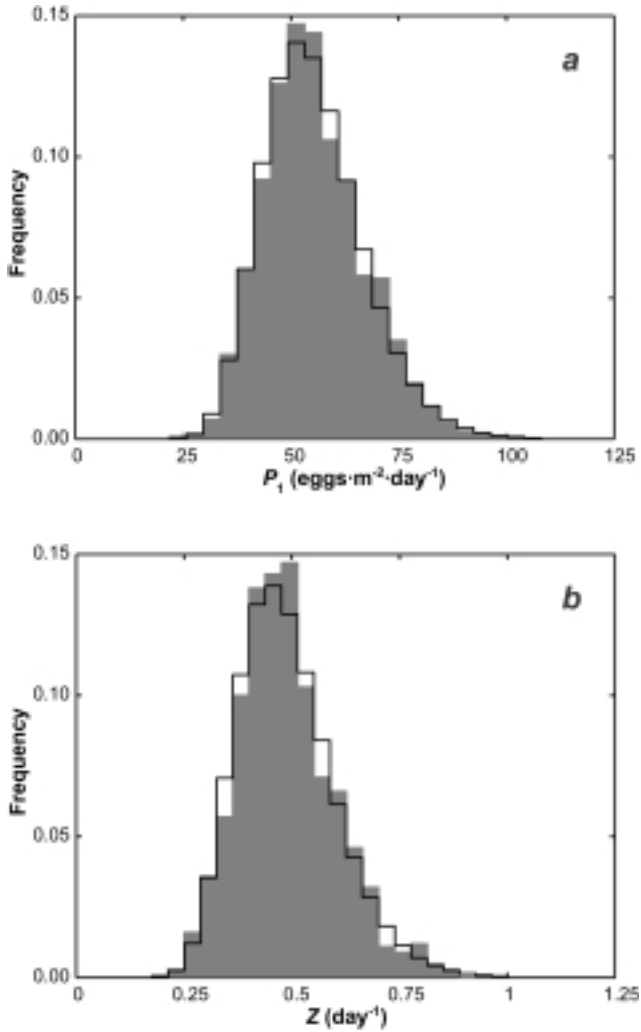


Fig. 4. Empirical marginal probability distribution of P_1 (a) and Z (b). Solid line: expected log-normal frequency; shaded area: observed frequency. The model is the same as in Fig. 2. The log-normality hypothesis was confirmed by a Kolmogorov-Smirnov test for both P_1 ($d = 0.0182$, $p > 0.2$) and Z ($d = 0.0204$, $p > 0.2$).

tive population and the fraction of females spawning per day must not fluctuate widely. This aspect was never stressed in previous works, nor was the agreement of data to the hypothesis properly assessed; this is of fundamental importance and deserves future research. In conclusion, we believe the DEPM can be an effective method for the stock assessment of multiple spawning pelagic fishes, but further work is required to establish the actual reliability of DEPM biomass estimates.

Acknowledgements

We thank Nancy C. H. Lo (Southwest Fisheries Center, La Jolla, USA) and an anonymous reviewer for useful suggestions. This work was partially funded by Ministero delle Politiche Agricole, Direzione Generale della Pesca e dell'Acquacoltura, and the Commission of the European Communities, Directorate General for Fisheries XIV.

References

- Caragitsou, E., N. Casavola, V. Vassilopoulou, A. Siapatis, A. Anastasopoulou, G. Christides, E. Hajdëri, E. Rizzi & A. Aprea, 2001: Estimation of the Mediterranean anchovy (*Engraulis encrasicolus*) biomass by the daily egg production method in the Thracian Sea (Greece) and South Western Adriatic Sea (Italy). Final Report for the EC Project 98/040.
- Dennis, B., P. L. Munholland & J. M. Scott, 1991: Estimation of growth and extinction parameters for endangered species. *Ecol. Monogr.* **61**(2): 115–143.
- Efron, B., 1979: Bootstrap methods: another look at the jackknife. *Ann. Statist.* **7**: 1–26.
- Efron, B., 1982: The Jackknife, the Bootstrap and other resampling plans. *CMBS-NSF Comb. Ser. Appl. Math.* SIAM No. 38.
- Lo, N. C. H., 1985: A Model for Temperature-Dependent Northern Anchovy Egg Development and an Automated Procedure for the Assignment of Age to Staged Eggs. In: R. Lasker (Ed.), *An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, Engraulis mordax*. NOAA Tech. Rep. NMFS **36**: 43–50.
- Lasker, R. (Ed.), 1985: *An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, Engraulis mordax*. NOAA Tech. Rep. NMFS **36**.
- Hunter, J. R., & N. C. H. Lo, 1997: The daily egg production method of biomass estimation: Some problems and potential improvements. *Ozeanografika* **2**: 41–69.
- Moser, H. G. & E. H. Ahlstrom, 1985: Staging Anchovy Eggs. In: R. Lasker (Ed.), *An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, Engraulis mordax*. NOAA Tech. Rep. NMFS **36**: 37–41.
- Motos, L., 1994: Estimación de la biomasa desovante de la población de anchoa del Golfo de Vizcaya, *Engraulis encrasicolus*, a partir de su producción de huevos. Bases metodológicas y aplicación. Ph. D. Thesis. Universidad del País Vasco; 240 pp.
- Palomera, I., 2001: The Application of the Daily Egg Production Method for Spawning Biomass at the Mediterranean Sea: Overview. *Rapp. Comm. Int. Mer Médit.*
- Picquelle, S. & G. Stauffer, 1985: Parameter Estimation for an Egg Production Method of Northern Anchovy Biomass Assessment. In: R. LASKER (Ed.), *An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, Engraulis mordax*. NOAA Tech. Rep. NMFS **36**: 7–16.
- Regner, S., 1985: Ecology of planktonic stages of the anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the central Adriatic. *Acta Adriat.* **26**(1), Series Monographiae **1**: 1–113.
- Stauffer, G. & S. Picquelle, 1980: Estimates of the 1980 spawning biomass of the subpopulation of northern anchovy. *Natl. Mar. Fish. Serv. Southwest Fish. Cent., La Jolla, CA. Admin. Rep. LJ-80-09*; 41 pp.
- Uriarte, A. & L. Motos, 1998: Sampling errors in anchovy egg abundance estimates using the PAIROVET net. *J. Plankton Res.* **20**: 1861–1888.