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EXPERIMENTAL INVESTIGATIONS ON THE EFFECT OF TEMPERATURE ON EGG PRODUCTION, LARVAL QUALITY AND RECRUITMENT IN FLATFISH

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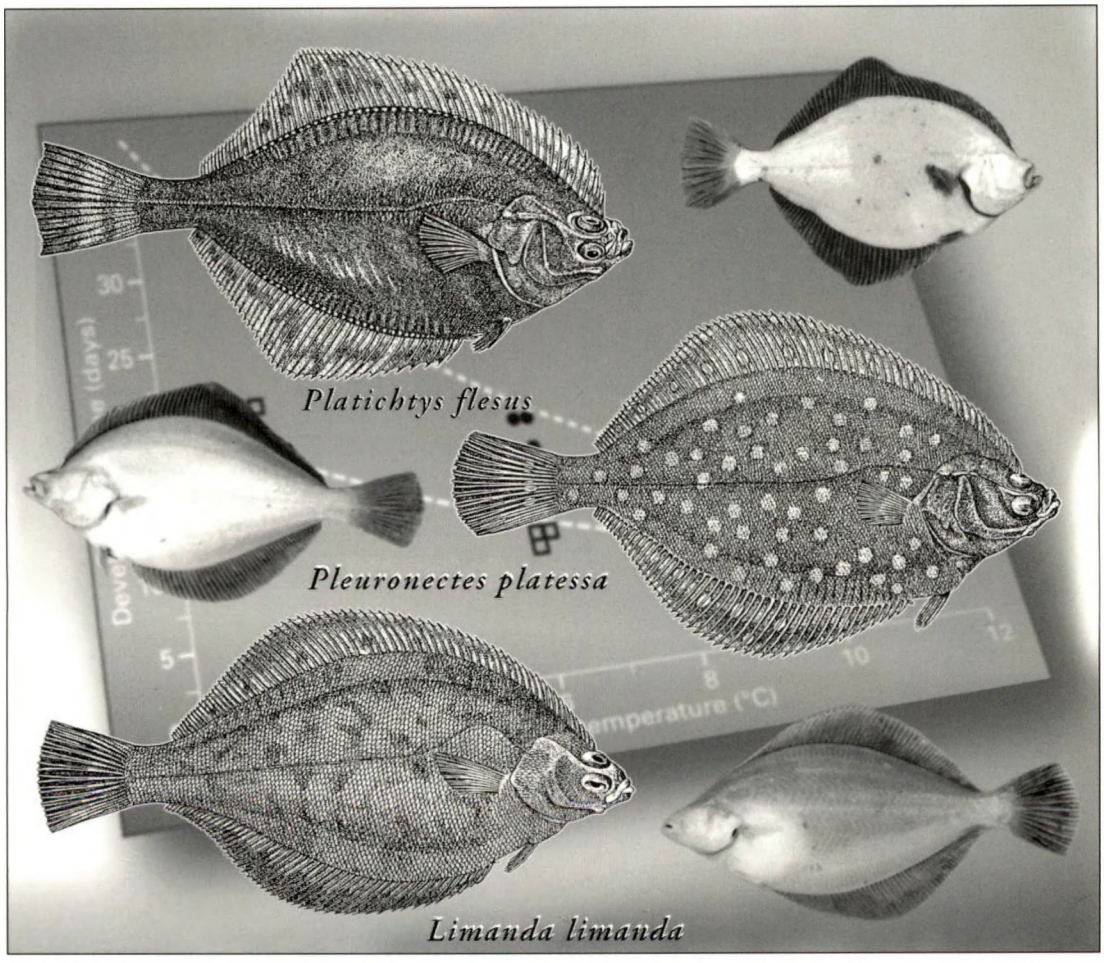

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EXPERIMENTAL INVESTIGATIONS ON THE EFFECT OF TEMPERATURE ON EGG PRODUCTION, LARVAL QUALITY AND RECRUITMENT IN FLATFISH

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SAMENVATTING

De jaarklassterkte bij schol *(Pleuronectes platessa*) lijkt mede bepaald te worden door de wintertemperatuur: een warme winter resulteert meestal in een zwakke jaarklasse, terwijl een strenge winter meestal resulteert in een sterke jaarklasse. In dit rapport worden met behulp van laboratoriumexperimenten 3 mogelijk verklaringen onderzocht voor deze relatie: [1] het effect van temperatuur op de totale eiproduktie bij schar (*Limanda limanda*) en bot (*Platichthys flesus*). De eiproduktie van schar en bot werd bepaald bij constante temperaturen van 2, 6 en 10°C. De eiproduktie was het hoogst bij 2 en het laagst bij 10°C. [2] het effect van de incubatietemperatuur van de eieren op de groei en meristische variatie van juveniele schol. Scholeieren zijn geïncubeerd bij 2, 6 en 10°C, en de larven werden opgekweekt bij 10°C. De groeisnelheid en de aantallen wervels en vinstralen van de juvenielen werden bepaald bij 5 en 20°C. De incubatietemperatuur had geen effect op de groeisnelheid en er was geen relatie tussen de groeisnelheid en de aantallen wervels en vinstralen. [3] het effect van de incubatie temperatuur van de eieren op de lengte van botlarven. De incubatie temperatuur van de eieren had effect op de lengte van botlarven: na incubatie bij lage temperatuur waren de larven kleiner dan na incubatie bij hoge temperatuur. Op het moment dat de larven beginnen te eten bestond dit verschil niet meer. Schar, bot en schol paaien in een seizoen meerdere malen. Schar produceert kleine legsels met kleine eieren, bot produceert grote legsels met kleine eieren terwijl schol grote legsels produceert met grote eieren. Deze verschillen worden bediscussieerd met betrekking tot de ligging van de leefgebieden van de juvenielen, de paaiperiode en de eetgewoontes tijdens de paaiperiode.

SUMMARY

Year-class-strength in plaice *(Pleuronectes platessa)* appears to be determined by winter temperature. A high winter temperature usually results in a weak year-class, whereas a cold winter usually results in a strong year-class. In this report three possible mechanisms to explain this variation have been studied in laboratory experiments: [1] the effect of temperature on egg production in dab (*Limanda limanda*) and flounder (*Platichthys flesus*). Egg production of adult dab and flounder was estimated at three constant temperatures of 2, 6 and 10°C. Egg production was high at 2°C and low at 10°C. [2] the effect of egg incubation temperature on juvenile growth and meristic variation in plaice. Plaice eggs were incubated at 2, 6 and 10°C and the larvae were all reared at 10°C. The obtained juveniles were examined for differences in growth rate at 15 and 20°C and for meristic variation. Egg incubation temperature did not have an effect on the growth capacity of the juveniles and there was no relationship between growth rate and the number of vertebrae and fin rays. [3] the effect of incubation temperature on length of larval flounder. Incubation temperature did affect the length of flounder larvae at hatch, but did not affect the length at the first-feeding stage. Dab, plaice and flounder are 'repeat spawners', with different reproductive strategies. Dab produces small batches, with small eggs, flounder produces large batches, with small eggs, whereas plaice produces large batches, with large eggs. These differences are discussed with respect to the position and size of the nurseries, the timing of spawning and the feeding behaviour of the adults during the spawning period.

1. INTRODUCTION

Plaice *(Pleuronectes platessa)*, dab *(Limanda limanda)* and flounder *(Platichthys flesus)* are abundant flatfish in the North Sea and the Wadden Sea.

Plaice is found to a depth of 80 metres in the North Sea. The main nurseries for populations in the southern North Sea are located off the coast of Great-Britain and in the Dutch, German and Danish Wadden Sea. For spawning, plaice migrates to special spawning areas in the German Bight, the southern North Sea and the English Channel, where they spawn from December until March (HARDING *et al.*, 1978). Adult plaice stop feeding during the spawning period (RIJNSDORP, 1989). This means they have to build up reserves in summer, which are used for reproduction and maintenance in winter (RIJNSDORP, 1990). Fecundity is high, a female can produce 20,000-300,000 eggs in a spawning period (BANNISTER, 1978). Total egg production of the plaice populations in the southern North Sea is estimated at 16 - 21×10^{12} eggs (HEESEN & RIJNSDORP, 1989). The development time to various embryonic stages shows an inverse relationship with temperature (RYLAND *et al.*, 1975), the eggs hatch within several weeks. The pelagic eggs and larvae are transported by the residual tidal current in a north-easterly direction, with a velocity of 2-6 $km.d^{-1}$. In this period the symmetric plaice larvae eat zooplankton. After several weeks the process of metamorphosis starts: the left eye moves over the side of the head and the larvae obtain the asymmetrical flatfish shape. During metamorphosis the larvae migrate towards shallow coastal areas where they stay close to the bottom and finally settle on their nursery grounds (RIJNSDORP *et al.*, 1985). The fish find shelter and food on the nursery grounds for one or two years. After this period they leave the area to stay in the North Sea. Males may become mature at age two or three, females at age four or five (RIJNSDORP, 1989).

The spawning area and period for flounder are also well studied. Flounders spawn from February to May in an area west and northwest of the Dutch west coast, in the eastern English Channel and in the area northwest of Helgoland (MUUS & DAHLSTRÖM, 1978, VAN DER LAND, 1991). They do not eat during the spawning period. The pelagic eggs and larvae are transported to the nursery grounds in the same way as for plaice. The juvenile flounders use the same nurseries as plaice (VAN DER VEER *et al.*, 1991), freshwater and estuarine areas are also frequently used (BERGHAHN, 1984). Unlike plaice, flounders stay in the coastal areas, where they can reach the adult size of 30-50 cm.

Unlike plaice and flounder, dab eat during the spawning season. Dab may spawn from January to September, with major spawning activity in the North Sea from February to April. Spawning occurs throughout the south-eastern North Sea with offshore concentrations of eggs in the German Bight, north of the Frisian Islands, along the southern edge of the Dogger Bank and northeast of Flamborough Head (VAN DER LAND, 1991). The eggs and larvae are pelagic and show a similar development to plaice and flounder. After metamorphosis the larvae settle at a length of 13-20 mm. Dab use the whole southern North Sea as a nursery ground, but they settle particularly in coastal areas (BOLLE *et al.*, 1994).

Recruitment mechanisms of flatfish, especially plaice and flounder have been investigated for many years and some factors determining year-class strength have been specified. Recruitment in plaice and flounder appears to be mainly determined during the embryonic pelagic and early juvenile stages (VAN DER VEER, 1986; VAN DER VEER *et al.*, 1990; 1991). Winter temperature appears to be an important factor influencing recruitment: in plaice a cold winter usually results in a large year-class (BANNISTER *et al.*, 1974, ZIJLSTRA & WITTE, 1985, VAN DER VEER, 1986).

The question arises why cold winters might lead to higher numbers of settling plaice larvae in the nurseries. There are several possible explanations why a cold winter might be followed by a strong year-class:

[1] Temperature may affect the activity and the energy requirements of the predators of fish eggs and larvae. Little is known about predation on plaice eggs. Invertebrate predators are not abundant at the time of egg development, but the herring *(Clupea harengus)*, overwintering in the southern part of the North Sea, has been identified as a possible predator (POMMERANZ,

1981). Possibly coelenterate predation is important for larval plaice and flounder (VAN DER VEER, 1985). Another possibility is that temperature affects growth rate of pathogen bacteria, and so influences the survival of fish eggs.

[2] Temperature also has a direct effect on the mortality, rate of development of eggs and growth rate of the larvae. Mortality of eggs and larvae is correlated with temperature (HARDING *et al.*, 1978, ZIJLSTRA & WITTE, 1985). Incubation temperature of the eggs also has an effect on the size of the larvae: at higher temperatures plaice larvae are smaller (RYLAND *et al.*, 1975). In *Coregonus lavaretus* an optimum incubation temperature with maximal larval sizes was found at 4°C (OSIETZKI, 1991). Swimming speed of plaice larvae is correlated with temperature but also with larval size (RYLAND, 1963). This means that larger larvae have better opportunities to escape from predators (BAILEY, 1984, reviewed by BAILEY & HOUDE, 1989). SHELBOURNE (1962) found a predator-prey size relationship for plaice larvae feeding on *Oikopleura*: bigger larvae have an advantage because they have a bigger gape width, therefore they can eat bigger prey and obtain more food. All temperature effects on processes of mortality and growth of the larvae may influence recruitment.

[3] Low winter temperatures may influence the energy requirements of the adult spawning stock. If temperatures are low the adults need less energy for their maintenance, and therefore have more energy left for producing eggs, more eggs leading to a stronger year-class. HARDING *et al.* (1978) found a correlation between stage V egg abundance in the North Sea and the winter temperature, while BANNISTER (1978) observed a correlation between egg production and abundance of stage V-eggs. The missing link between these two observations is the relation between winter temperature and egg-production. Temperature may have an effect on the total egg production, but also on the length of the spawning period.

[4] Finally egg incubation temperature may also have an effect on the quality of larvae and juvenile fish. It is known that incubation temperature of the eggs influences the number of vertebrae and fin rays in fish (MOLANDER & MOLANDER-SWEDMARK, 1957; TÄNING, 1944; FONDS *et al.*, 1973, review by LINDSEY, 1988). Whether incubation temperature of the eggs influences the optimal growth temperature or the growth capacity and survival of the 0-group plaice is not known. There may be irreversible non-genetic adaptation. KINNE (1962) concluded on the basis of salinity adaptation experiments with the fish *Cypronodon macularius*, that certain ecophysiological optima are fixed in early development, in much the same way as meristic characteristics are fixed by temperature during the embryonic development. Thus KINNE (1962) suggests that an individual fish will grow better at salinities near the salinity at which it passed its own critical developmental phase. In our case, the question arises whether different temperatures during embryonic development, may result in different temperature optima for growth of the young fish and so in a correlation between fin rays, vertebrae and growth rate. In plaice both size-selective mortality and a size-selective onset of maturation have been found. Hence faster growth in the juvenile stages may result in earlier, and so higher, recruitment (RIJNSDORP, 1993).

The aim of this study was to test how winter temperature can affect recruitment in flatfish, by testing the possible importance of hypotheses 2 to 4 in laboratory experiments with the following direct questions:

1. Does incubation temperature of the eggs affect the optimum growth rate of the juvenile fish and is there any relation between growth rate and meristic characters (vertebrae and finrays)?
2. Does winter temperature influence the total egg production of the female fish?
3. What is the effect of egg incubation temperature on the length of the larvae?

2. METHODS

Experiments on reproduction of plaice, flounder and dab were carried out at temperatures of 2, 6 and 10°C, representing a very cold, an average and an extremely warm winter in the southern North Sea. Seawater was obtained from the Marsdiep, a tidal inlet of the Wadden Sea, and stored in large tanks, with sand filters and a water recirculation system.

2.1. GROWTH RATE AND MERISTIC VARIATION IN JUVENILE PLAICE

Adult plaice were collected with a beam-trawl on 19 December 1994 at the Broad Fourteens and the Dogger bank in the North Sea. They were stored in the laboratory in a tank of 2.5x0.5x0.5 m with S=30 seawater at 9°C. Temperature was gradually lowered to 3°C and was kept at this level until the fish stopped spawning at the end of the experiment. The tanks were provided with a seawater supply, aeration and a thin layer of sand on the bottom. To identify individual fishes, some were kept separately in a perforated plastic crate (50x35x25 cm), hanging in the tank.

2.1.1. EGG INCUBATION

Flatfish such as plaice, flounder and dab do not spawn naturally in small tanks and because we wanted to know which adults produced eggs, eggs had to be stripped from the fish. Mature female plaice were stripped when they appeared to be ripe. Ripe eggs were collected in a beaker and the sperm of male plaice was added immediately. Eggs and sperm were left in the beaker for 20 min. and then transferred to a 1 litre glass beaker with seawater at a salinity of S=35-36. At this salinity healthy eggs float and dead eggs sink to the bottom. After 30 min. the floating eggs were collected in a 10 litre bucket with S=35-36 at 6°C. The water was aerated gently and antibiotic solution was added, 25.000 International Units of Penicillin and 25 mg Streptomycin per litre seawater (RYLAND, 1966).

The eggs were left overnight at a common temperature of 6°C to allow for proper fertilization and a start of development. The next morning the batch was divided over three pails and incubated at 2, 6 and 10°C respectively. Dead eggs were siphoned from the bottom at least three times a week and the water in the bucket was changed approximately once a week, according to temperature. Temperature was measured daily and adjusted when necessary. The eggs were incubated until they hatched and the larvae were kept at the three egg incubation temperatures until they had developed ocular pigments (first feeding stage).

2.1.2. LARVAL REARING

After the larvae had reached the stage of first feeding, they were transferred to green polyester-fibreglass rearing tanks (50x45x50 cm) with seawater of S=30. Larvae were grown to metamorphosis at a common rearing temperature of 10°C because it would take too long to grow them to metamorphosis at 2 and 6°C. Antibiotics were added to the rearing tanks in the same concentration as for the eggs. It appeared that plaice larvae did not start feeding without an extra supply of light, therefore the tanks were constantly illuminated with two 36 W/82 tube-lights.

The larvae were fed with zooplankton collected in the Marsdiep. Plankton was fished daily with a 100 and a 200 µm mesh-size net. The nets were hung for one hour in the tidal current from a short jetty, usually with the incoming tide. The plankton catch was sieved through a 2 mm mesh-size to take out the coelenterates and other unwanted larger plankters. In order to get rid of the phytoplankton the catch was divided over several 25 litre buckets with sea water and left overnight. The next day, most of the phytoplankton had settled on the bottom of the bucket and the zooplankton was sieved over a 200 and a 100 µm net. Zooplankton from the 200 µm net was sieved again through a 800 µm gauze to remove the shrimp-larvae and other large plankters. What remained were portions of fine and coarse zooplankton. The former was fed to the young larvae, the latter to the elder larvae.

Dead larvae and other particles settling on the bottom of the rearing tanks were removed regularly. Water was only changed when it was necessary, e.g. when it started smelling bad.

2.1.3. JUVENILE GROWTH

The aim was to determine the effect of egg incubation temperature on the growth capacity of the juveniles. Therefore the maximal growth rate was determined for different groups of young plaice at two temperatures of 15 and 20°C.

Growth experiments with the young plaice were started when all larvae of one sub-batch had metamorphosed and the fish measured between 11 and 30 mm total length. Growth rate of juvenile plaice was measured at 15°C and 20°C in the same green, polyester-fibre tanks as used for rearing of the larvae. The two temperatures represented the mean water temperature conditions in the Wadden Sea

during a cool and a warm summer. The rearing tanks were supplied with running seawater (approximately 2.5 $l.h^{-1}$) and were aerated. A thin layer of sand was put on the bottom, since young flatfish without sand often suffer from bacterial diseases such as *Vibrio* or 'black patch necrosis' (cf. McVicar & White, 1982). Ten fish were reared in each of these tanks. The rest of the fish, if there were not more than 25, were reared in black 100 litre tubs at room temperature (13 to 22°C). These tubs were aerated, but without water circulation. Water was changed once every two weeks.

Growth rate was estimated in $mm.d^{-1}$. At the start of the growth experiment, the length of the metamorphosed fish (L) was measured to the nearest mm total length. The young plaice were distributed over 3 different tanks, so that fish in all tanks had a similar length distribution. They were fed to satiation daily with chopped fresh mussel meat. Fish that died during the experiment were, if they were discovered before they had disintegrated, measured and stored in 70% ethanol. After 28 days (4 weeks) the length of the young plaice was measured again. They were killed with MS 222 (Sandoz) and measured to the nearest mm total length. Daily growth of individual fish was estimated as:

$$\text{Daily growth } (mm.d^{-1}) = \frac{L_{end} - L_{begin}}{28 \text{ days}}$$

The young plaice were sorted according to their length, assuming that the length distribution did not change much from beginning to end of the growth experiment. For comparison of growth rates the growth has to be compensated for length. Fish that died have not been included in the estimates of daily growth. After measuring all plaice were preserved in 70% ethanol for further analysis of meristic characters (vertebrae and fin ray numbers).

2.1.4. MERISTIC CHARACTERS

Numbers of vertebrae and fin rays of the juvenile plaice were counted after staining with alizarin (Täning, 1944). The urostyle was counted as one vertebra. The staining method was adapted according to Seigle & Jones (1995), see Appendix 1. The fish were sorted according to length and numbers of vertebrae were compared with growth rate. The numbers of fin rays of the parent fish were counted and also the number of vertebrae after removal of the meat.

2.2. FECUNDITY AT DIFFERENT TEMPERATURES

Egg production was determined by stripping the eggs from adult fish that were kept at 3 different constant temperatures of 2, 6 and 10°C over a period of several months.

2.2.1. LABORATORY CONDITIONS

The fish were kept in temperature controlled tanks that measured 2.5x0.5x0.5 m., with a supply of S=30 seawater and continuous aeration. The tanks had a layer of 1 to 3 cm of sand on the bottom. The fish were kept under natural daylight conditions due to glass windows in the roof of the laboratory. The tanks were partially covered in order to prevent the fish from jumping out and to reduce illumination. The fish were fed till satiation one to three times a week with fresh mussel meat (*Mytilus edulis*), defrosted shrimps (*Crangon crangon*) and young herring (*Clupea harengus*). The dabs fed well during the whole spawning season, the flounders refused any food.

2.2.2. THE FISH

Dab *(Limanda limanda)* were caught in the North Sea with a beam-trawl along the coast of North Holland (Callantsoog) on 1 December 1994 and another 60 adults were collected on 19 December 1994 at the Broad Fourteens and the Dogger bank. All males were stored in one tank at 5.0±2.0°C. The males did not mature and therefore were not used in the experiment. The females were sorted into equal groups (in numbers and size) and divided over 3 different tanks. Water temperature in these tanks was adapted gradually from 9°C to 2, 6 and 10°C respectively.

About 48 flounders were caught by a shrimp fisherman in the Wadden Sea on 30 November 1994 and another 8 fish were collected on 10 December at Callantsoog. They were stored for 2 weeks at 10°C. In this period about 25 flounders died, probably due to rough handling and storage on board of the shrimp trawler. The survivors were sorted into 3 approximately equal groups (in numbers, size and sex), which

were stored in 3 tanks and gradually adapted to temperatures of 2, 6 and 10°C respectively.

2.2.3. EGG PRODUCTION

Eggs from dab and flounder were stripped when they were judged ripe. This was about 3 times per month for dab. With flounder stripping was tried about twice a week at 10°C and 6°C and once a week at 2°C. Dab were very active, and were therefore lightly anaesthetised with MS 222 (Sandoz) before stripping the eggs. Eggs were collected in a petri-dish and weighed. A subsample was weighed and counted to determine the total number of eggs in a batch. Only viable eggs were counted. This means that broken eggs, empty egg shels and undeveloped eggs were not included in the counts. The fish that produced eggs were identified by their length, width, weight, left/right eyed side in flounder and other distinguishing features such as spots and scars. After the eggs were stripped, each fish was stored individually in another tank at the same temperature. When it was stripped again, after one or two weeks, the fish was put back in it's original tank. This helped to identify the individual fish and their total production of egg batches.

The fish were weighed to estimate the egg production per gram fish. Unfortunate the weights were not measured before the spawning period. Therefore the weight of the fish after production of the first batch was used to estimate eggs per gram fish.

Fish that stopped spawning or died were removed from the tank, measured, identified and returned or discarded to the sea.

2.3. DEVELOPMENT OF FLOUNDER IN RELATION TO TEMPERATURE

The aim was to determine the effect of incubation temperature on the rate of development and the length of flounder larvae at hatch and at first feeding. The influence of temperature on length of the larvae was estimated by incubating eggs from a female at the same temperature at which the female ripened. Length of the larvae was measured at hatch and at first feeding.

Eggs that were stripped as described in 2.1.1 and 2.2.3, were fertilised with sperm from one or two males. When available, a male from the same temperature was used as the egg producing female. After artificial fertilization the eggs and sperm were left in the cup for 20 min. Then they were put in a 1 litre glass beaker with salinity of S=35-36. At this salinity healthy eggs float while dead eggs sink to the bottom. After half an hour the floating eggs were collected in another 1 litre glass beaker with clean seawater of S=35-36 and stored at the proper incubation temperature. Antibiotics were added (see 2.1.1.) and the beakers were gently aerated. Seawater was changed at least once a week and dead eggs were removed regularly by siphoning.

When 90% of the larvae had hatched, the total length of 10 larvae was measured with a binocular-micrometer. The measurement was repeated with a sample when they had reached the stage of first feeding. The first-feeding stage was defined as the stage when the larvae are able to start feeding. The typical features of this stage are fully pigmented (black) eyes, a fully developed mouth and the active swimming of the larvae in a normal upright position

2.4. STATISTICS

Statistical test were carried out with Systat 5.1 for Windows. Differences were tested with an ANOVA and a Tukey HSD-test at p=0.05.

3. RESULTS

The actual temperature measurements in the experiments are presented in Tables 2 and 6 and Figure 10. In this report the subsamples of egg batches are coded by their spawning date, egg incubation temperature and rearing temperature for the larvae. For example P1403-6-10 means, plaice spawned on 14 March, eggs incubated at 6°C and larvae reared to metamorphosis at 10°C.

3.1. GROWTH RATE AND MERISTIC VARIATION IN JUVENILE PLAICE

Several batches were stripped from ripe female plaice, fertilized and incubated at 2, 6 and 10°C. Mortality of the larvae reared at 10°C was often high and only one batch, stripped and fertilized on 14 March, produced metamorphosed young flatfish for all 3 egg incubation temperatures. Due to a high egg and larval mortality, in the 2°C subsample only four metamorphosed fishes were obtained. To compensate for this low number, another 2°C subsample from the same parent fish, obtained on 27 March, was used as

a duplo. Larvae from the other subsamples from this batch, incubated at 6 and 10°C during egg development, did not survive to metamorphosis.

In P1403-10 so many larvae hatched, that half of them were grown in another tub at room temperature of 14.3±1.30°C. Metamorphosed young fish reared in this tub were also examined for growth rate and meristic characters. Numbers of eggs and larvae in subsamples from one of the two batches used are presented in Table 1. When the initial number of eggs is compared with the ultimate number of fish produced for the growth experiment, it becomes clear that egg and larval mortality were very high.

3.1.1. GROWTH EXPERIMENTS

Table 2 shows the results of the growth experiments. Length distribution of the young plaice within and between subsamples was not equal. Since growth rate of young plaice depended on initial length (Fig. 1), the mean growth rates could not be compared without correction for length. The growth rate per mm initial length was a better measure for comparison of the growth capacity of the juvenile reared from eggs incubated at different temperatures.

The growth rate per mm initial length at 15°C is shown in Fig. 2: incubation temperature had a significant effect on growth rate of young plaice at 15°C (ANOVA, p=0.011). The growth rate of fish from eggs incubated at 2°C was significantly lower than the growth rate of fish from eggs incubated at 10°C (Tukey HSD, p=0.012), and almost significantly different from the growth rate of fish from eggs incubated at 6°C (Tukey HSD, p=0.068). However, the growth rate per mm initial length at 20°C (Fig. 3) was not correlated with incubation temperature. The growth rates at 20°C were equal to growth rates at 15°C, except for fish from egg incubated temperatures of 2°C. For these fish the growth rate appeared to be higher at 20°C than at 15°C. Growth rates measured at room temperature were of little use, because often too many fish died, some probably because of lethal temperatures (P2703-2-10, temperatures of 23°C). Since room temperature varied consideraly during the different experiments, growth rates could not be compared.

In several of the growth experiments some fish died, and disappeared before the end of the growth experiment. At 15°C no fish died, but at 2°C and at room temperature often a substantial number of the fish died during the experiment. This may have resulted in an overestimate of growth rates, because mortality may have been higher for the slow growing animals.

3.1.2. MERISTIC VARIATION

The meristic characters of the parent fish of all reared juvenile plaice are presented in Table 3. The estimated numbers of vertebrae and fin rays in the different groups of young plaice reared at one common temperature of 10°C, but originating from subsamples of eggs incubated at 2, 6 and 10°C until the stage of 'first feeding' larvae, are presented in Table 3.

Egg incubation temperature had an effect on vertebral number (ANOVA, p=0.000, Table 3, Fig. 4). Both high (10°C) and low (2°C) egg incubation temperatures resulted in a higher mean number of vertebrae, while an intermediate temperature (6°C) resulted in a lower mean number of vertebrae. The vertebral counts for egg incubation temperatures of 2.4 and 6.1°C were significantly lower than those of 10.3°C (Tukey HSD, p=0.029). Rearing temperature of larvae did not have an effect on vertebral count. This could be concluded from the comparison of P1403-10-10 with P1403-10-14. Fish from these batches had the same mean vertebral number. This means that the number of vertebrae was already determined in the egg stage or the early larval stage and that for comparison of the numbers of vertebrae the data from P1403-10-10 and P1403-10-14 could be combined. Rearing temperature of the larvae did affect the number of fin rays, indicating that number of fin rays was not completely determined at hatch. This became clear by comparison of P1403-10-10 with P1403-10-14 (Table 3). The larvae reared at 14°C had higher dorsal and anal fin ray numbers, than those reared at 10°C. Because P1403-10-14 had a higher fin ray count, the data could not be combined with P1403-10-10.

Dorsal and anal fin rays both showed the same response to incubation temperature of the eggs, an Λ-shaped (arched) curve (Figs 5 and 6). The number of fin rays for the larvae from

P1403-10-14 was high compared to the larvae from P1403-10-10. The range of the differences of the means was small. The total variation range for numbers of dorsal fin rays was 11 fin rays while the maximal difference between the mean was 1.05 fin rays. The total variation range for anal fin rays was 8 fin rays while the maximal difference between the mean was 1.44 fin rays. The most frequent number (mode) of anal fin rays showed a slight linear increase with egg incubation temperature (Fig. 6). P1403-10-14 again showed that transferring the first feeding larvae for rearing to a higher temperature at hatch increased the number of fin rays.

No correlation between numbers of vertebrae and anal fin rays was found. However, there appeared to be a positive correlation between vertebrae and dorsal fin rays (ANOVA, p=0.045) and also between dorsal and anal fin rays (ANOVA, p=0.000). There was a non-significant relation between the growth rate and the number of anal fin rays: Growth rate ($mm.d^{-1}$) = 0.0018*Anal fin rays -0.26; F=2.12; n=117. The relation between rearing temperature of the larvae and the number of fin rays, indicated that there might be a relation between the growth capacity of juvenile plaice and the rearing temperature of the larvae.

3.2. FECUNDITY AT DIFFERENT TEMPERATURES

The results of estimates of total egg production of plaice, flounder and dab are presented in Table 4. They indicated that the three flatfish species had completely different reproductive strategies. Plaice produced 2 to 5 batches per fish with large eggs (maximal 0.1 x 10^6 eggs/fish), the flounders produced 3 to 10 large batches per fish with small eggs (maximal 1.1 x 10^6 eggs per fish), whereas the dabs produced 3 to 7 small batches per fish with small eggs (maximal 0.1-0.2 x 10^6 eggs per fish). The number of batches and the spawning period were highly dependent on temperature in all three species. The total egg production was highest for flounder, intermediate for plaice and low for dab.

3.2.1. FLOUNDER

The total egg production in flounders was related to the body weight of the fish (Fig. 7). Because the weight distribution of the flounders kept at the different temperatures was not equal (Table 5), the total egg production at the different temperatures was corrected for body weight of the fish. Egg production of flounders at 2, 6 and 10°C was different in amount and timing (Fig. 8). At 10°C the egg production was low and restricted to a short period, whereas at 6°C the egg production was high during a similarly short season. The season at 2°C was prolonged into July, but the egg production per week was low (Fig. 8). At 10°C several fish died before they stopped reproducing, probably due to handling (See Appendix 2). Fish that spawned less then three times, or died before they stopped spawning and did not produce more than 2 batches, were not used for the estimation of total egg production (Table 5). They are marked with an * in Appendix 2. It should be noted that weight of the eggs produced was not a good measure for fecundity, because the water content of batches was variable.

The data from Appendix 4 are summarized in Table 5. A clear trend of higher egg production at lower temperatures was obvious. Total egg production per gram fish and gram egg production per gram fish were significantly higher at 2 and 6°C than at 10°C (ANOVA, p=0.03 and p=0.006). The spawning period and the number of batches per fish were significantly higher at 2°C (ANOVA, p=0.001 and p=0.006). There was no statistically significant difference in the number of eggs per batch at the different temperatures.

3.2.2. DAB

In Table 6 the results of the egg production of dab are presented. Egg production was also corrected for body weight of fish. Dab which did not spawn, have not been included in further calculations. It should be noted that standard deviations of the means were very high in this experiment, so it was not possible to use an ANOVA.

Egg production at 10°C could not be used in this comparison because too many fish contracted a disease and died at this temperature (see Appendix 5). Due to disease and mortality only 3 out of 21 fishes produced eggs at 10°C. Egg production at 2°C was higher than at 6°C. This was mainly due to the higher number of batches per fish at 2°C and

differences in batch size. Batches were at least twice as large at 2°C as compared to 6°C.

3.3. DEVELOPMENT OF FLOUNDER IN RELATION TO TEMPERATURE

Glass beakers in which the eggs were incubated, were cooled by putting them in a crate in the fish tank. Water temperatures in the glass beaker varied with the temperatures in the fish tank, but were usually slightly higher than tank temperatures. Total lengths of the flounder larvae, hatched at different incubation temperatures, are presented in Fig. 9. Not every batch was measured at both hatch and first feeding, therefore the number of data points is not the same for these two stages. Numbers of batches examined at the different temperatures were not equal.

Length at hatch was dependent of temperature (ANOVA, $p=0.047$), with the smallest larvae at the lowest temperature. However, the length of flounder larvae at first feeding appeared to be independent of the incubation temperature (ANOVA, $p=0.320$). Measurements of the incubation time (D, days) to ≥90% hatch and to the stage of first feeding at three different temperatures are presented in Table 7 and Fig. 10. The development time was inversely related to temperature and decreased exponentially with temperature. The relationship between incubation temperature (T;°C) and the development time (D; days), could be described as:

a) hatch: $D=24.3e^{-0.1335T}$

b) first feeding: $D=45.8e^{-0.1483T}$

The data suggest that larval development after hatch of flounder slowed down more with decreasing temperature than egg development did.

4. DISCUSSION

4.1. GROWTH RATE AND MERISTIC VARIATION IN JUVENILE PLAICE

Incubation of plaice eggs at 2°C, resulted in lower growth rates of juvenile plaice at 15°C, but egg incubation temperature did not influence growth rates of the juveniles at 20°C. The fact that a different growth rate was found for only one subsample, makes it doubtful whether the observed growth rate was meaningful or just an artefact. The only factor which may have caused an artificial temperature effect is the higher mortality of the juvenile plaice at 20°C. If the observed temperature effect is real, this means that if a cold winter (2°C) is followed by a cool summer (15°C), growth of juvenile plaice will be low. This may possibly have an effect on recruitment and maturation of the fish (RIJNSDORP, 1993, VAN DER VEER *et al.*, 1994). Such a process would act as a recruitment-variability dampening factor, because it weakens a strong year-class, which arrives after a cold winter.

The growth rates obtained in this experiment, showed a different pattern as compared to the growth rates obtained by FONDS *et al.* (1992) for plaice. In our growth experiments growth rates at 15 and 20°C were equal, whereas FONDS *et al.* (1992) found higher growth rates at 20°C. The fish used in our experiments were small (1.2 to 3.5 cm) compared to the fish used by FONDS *et al.* (1992) (5 to 32 cm). Because growth of the juveniles is related to fish length, lower growth rates were expected in our experiment, but not found. Still, the effect of fish size on growth rate was obvious in our data, indicating that for newly metamorphosed plaice growth rate increases with increasing size.

Since year-class strength in plaice is mainly determined by February temperature (VAN DER VEER, 1986) and numbers of fin rays and vertebrae are determined by incubation temperature of the eggs and larvae (LINDSEY, 1988), these meristic characters might serve as a year-class strength index (FRANK, 1991). Incubation temperature does affect the mean number of vertebrae in plaice in a V-shaped curve (Fig. 4), but the difference between vertebral numbers for egg incubation temperatures of 2 and 6°C is only slight. MOLANDER & MOLANDER-SWEDMARK (1957) found a similar relation and variation between vertebral count and incubation temperature at incubation temperatures of 6, 8 and 10°C. The differences they found between the subsamples were in the same order of magnitude. DANNEVIG (1950) observed a negative relation between vertebral number and egg incubation temperatures of 4.93 and 5.41°C. This suggests that there is a negative relation between temperature and vertebral number between 2 and 8°C. Higher vertebral numbers were found at 10°C. For North Sea plaice eggs the winter temperatures range from 1.3-8.6°C and a negative relation between vertebrae and

winter temperature is expected. Together with the negative relationship between year-class strength and winter temperature, this suggests that a positive correlation between vertebral number and year-class strength of 0-group plaice may be found, in accordance with the hypothesis of FRANK (1991). In this respect the fin ray counts are less valuable, because the number of fin rays was not yet determined at hatch, when the larvae were transferred to 10°C. Data are lacking to test what effect this temperature break may have had on the ultimate number of fin rays, moreover this was beyond the purpose of this research. Fin rays appeared to be mainly related to larval rearing temperatures.

An important question is whether the differences observed here are due to a temperature effect on the formation of vertebrae, or the result of differences in selection on vertebral number due to selective mortality at different temperatures. Because mortalities were so high in this experiment, selection may have had a distinct effect on the mean vertebral number. TÄNING (1944) showed that a temperature effect on the formation of vertebrae and fin rays accounts for at least part of the differences. In his experiments with trout, mortality was negligible and still differences were found.

No direct or indirect relation between growth rate and numbers of vertebrae were found and there was no evidence that the number of vertebrae affects the fitness of a juvenile plaice. In larval stickleback *(Gasterosteus aculeatus)* the vertebral phenotype affects swimming speed and predation rates (SWAIN, 1992a, 1992b). Swimming performance was related more to the ratio of abdominal to caudal vertebrae (VR), than to the total number of vertebrae (VN). The optimal VR decreased as larval length increased. Unfortunately, we only counted the VN and not the VR. Experiments determining swimming speed and vertebral phenotype may reveal selection for these features. Variations in swimming speed and predation rate with vertebral number may explain changes during the season in mean vertebral number of 0-group plaice, as found in the field by BIES (1997). Therefore, selection on vertebral phenotype may be another way for temperature to affect the year-class strength. Remarkable is the (not-significant) correlation between growth capacity and the number of anal fin rays, indicating that there may be a relationship between growth rate and body structure.

4.2. REPRODUCTIVE STRATEGY

Plaice, dab and flounder are repeat spawners, they may spawn up to eight times over a period of several weeks. An interesting question is why dab, flounder and plaice show such different egg production strategies, whilst all three species are abundant and successful in the same area.

There appears to be a remarkable difference in lipid content between plaice eggs and eggs of dab and flounder. Plaice eggs have a fat content of only 1 to 2% of the dry weight whereas eggs of dab and flounder contain 5 to 10% lipid (pers. com. M. Fonds, eggs obtained from these experiments). Embryos use lipid for their energy requirements. Since plaice spawns early in winter, when water temperatures are low, the energy requirements for developing embryos are also low. Hence, less lipid is needed.

The difference in fecundity between plaice and flounder is easily explained. Flounders produce many small eggs and plaice produce fewer, but larger, eggs. Plaice embryos have to be bigger because they hatch earlier in the year, when prey is scarce and big (MCEVOY & MCEVOY, 1991). This means that fewer eggs can be produced, but the eggs produced have more reserves, hence mortality is probably lower and fewer eggs are needed.

The difference between flounder and dab cannot be explained in this way. The main difference in egg production is that dab produce fewer eggs; fecundity of flounders is 8-30 times higher. This suggests that mortality of egg, larval and juvenile stages is much higher in flounders. An important reason for this difference may be found in the use of nurseries by the 0-groups. 0-group dab use the whole North Sea as a nursery (BOLLE *et al.*, 1994), whereas juvenile flounder only use the Wadden Sea and the estuarine areas of rivers (VAN DER VEER *et al.*, 1991). This means that any dab larva that survives until metamorphosis is already in its nursery. Flounder larvae that do

not reach a nursery (with the residual tidal current) probably do not survive. There may be a relationship between the size (extension) of the nurseries and the fecundity of the species.

The location of the nursery may also explain why dab eat during the spawning period whereas plaice and flounder do not. Because the larvae of plaice and flounder have to reach restricted nursery areas with the tidal current, the location of the spawning areas are exactly defined. In these areas food is not necessarily abundant, but many fish are present during spawning. Food is possibly so scarce, that it is not economic to keep the digestive tract going. In contrast, immature plaice do eat in winter, but less than in summer (RIJNSDORP, 1989). Dab do not suffer from a lack of food since the whole North Sea can be used as a nursery, spawning may occur anywhere. They usually spawn later in the year, when food is more abundant.

4.3. EFFECT OF TEMPERATURE ON FECUNDITY

Plaice and flounder are closely related. Flounder is of little commercial interest and therefore its biology, (i.e. year-class strength and egg-production) has been less well studied than that of plaice. In the following discussion, plaice will sometimes be used as a model species for flounder, and vice versa.

The energy-budget of a fish can be described by the formula:

$$C = P + R + E$$

where C is consumption, P the production, R is the respiration and E are the excretion-products, faeces and urinal loss (BRAFIELD, 1985). R is higher at higher temperatures. In our case E is ignored, because E is proportional to C (dab) and to R (plaice and flounder) during the spawning period. In dab, that still feed during the spawning period, E is probably proportional to C. Because C = P + R and C=0 in plaice and flounder, the respiration rate of the female fish determines how large the (egg) production can be. If respiration is high, production will be low. In dab C>0, since dab eats during the spawning period, and P will be independent of R.

Fecundity of flounder, appears to agree with this formula. At high temperatures the total egg production is low and at low temperatures the egg production is high (Table 5). This is in accordance with field observations from BANNISTER (1978) in plaice. The variation (generated by temperature) found here, can explain half of the variation found in stage V egg abundance in plaice, as found by HARDING *et al.* (1978). Together with reduced egg mortalities during cold winters, egg production at different temperatures can explain a large part of the year-to-year variations in recruitment. The hypothesis from ZIJLSTRA & WITTE (1985) that adult egg production affects recruitment might be true, even when the adult stock size is equal in different years.

Adult stock size, together with the individual fecundity determines the total egg production and can possibly explain the variation which is not explained by individual fecundity. Unfortunately such data were not available.

The relationship between winter temperature and larval length of juvenile plaice is not only due to a slow development at low temperatures (ZIJLSTRA & WITTE, 1982; 1985), The low fecundity in dab cannot be explained with the energy budget formula. Because the dabs were fed till satiation, they were able to compensate for a higher respiration rates. This may also explain the later and prolonged spawning season, observed in this research (Fig. 8). A longer and later spawning season is not advantageous for flounder, because a coelenterate outburst in May may kill all late flounder larvae (VAN DER VEER, 1985). This may explain why there is a less clear relation between February temperature and the year-class strength in flounder as compared to plaice (Fig. 11). The effect of a cold winter on the timing of the coelenterate outburst is unknown.

The low fecundity of dab is not in agreement with the energy-budget formula. In the experiments the dabs were fed till satiation and they were able to compensate for higher respiration at higher temperatures. Hence egg production should be equal at different temperatures. Still egg production was higher at 2°C than at 6°C. There is no information available about the relation between year-class strength in dab and temperature during the spawning season. The results of the experiments presented here predict that an inverse relationship will be found, with cold winters resulting in strong year-classes.

4.4. DEVELOPMENT AND LENGTH OF FLOUNDER LARVAE

Incubation temperature affected the length at hatch of larval flounder, whereas temperature did not affect larval size at the first-feeding stage. Newly hatched larvae tended to be smaller after egg incubation at 2°C as compared to 6 and 10°C (Fig. 9). This is in agreement with observations from RYLAND *et al.* (1975) about the effect of temperature on the size of first feeding plaice larvae. RYLAND *et al.* (1975) found approximate equal lengths for plaice larvae at intermediate egg incubation temperatures (4 to 8°C), while at 10°C the larvae were much smaller. These results can be explained by a change in net yolk-conversion at different temperatures (RYLAND & NICHOLS, 1967). In plaice larvae, the growth in length per yolk disappearance shows an optimum between 6.5 and 8°C. At higher and lower temperatures growth in length per yolk disappearance was reduced. This suggests that at extreme high and low temperatures more yolk-energy is used for metabolism than for growth (RYLAND & NICHOLS, 1967). Another explanation for hatching of smaller larvae at low temperatures is that the larvae hatch at an earlier developmental stage at low temperatures. The ratio of development time to first feeding/hatch at 2°C is higher than at 6 and 10°C, so the larvae probably hatch in a earlier developmental stage at 2°C. It is not yet clear whether the general composition of the eggs produced at different temperatures were similar.

In the period between hatch and first feeding the larvae are smaller at low temperatures. In this stage of development flounder larvae are mainly inactive, they do not have fully developed eyes and they hardly respond to predators (BAILEY, 1984). Therefore it is not the swimming (escaping) speed which determines the predation rate in this period, but the chance of encounter. The encounter chance between fish larvae and predators is probably positively related to larval size, because larger larvae are easier to detect for the predators (LEGGETT & DEBLOIS, 1994). Optimal foraging theory predicts that predators will selectively pursue prey that maximises their energy cost-benefit ratio (reviewed in: STEPHENS & KREBS, 1986), and therefore will select the larger prey.

Since the length at first-feeding of flounder larvae is the same at all three temperatures (Fig. 9), the ability to escape from predators and to catch food will be the same at different egg incubation temperatures. If flounder larvae are smaller, predation may be less important. In this way cold winters with smaller larvae, may result in a stronger year-classes. Hence temperature may act as a variability generating factor. The relation between larval length and predation is not quantified. Therefore it is unknown which amount of variation in year-class strength may be explained by differences in larval length between years.

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TABLE 1

Plaice *(Pleuronectes platessa)*. Estimation of the number of eggs and other data for the egg-batches P1403 and P2703, used in the growth experiments.
* = larvae incubated at room temperature

Spawn date	14-Mar				27-Mar
Batch weigth (g)	219.04				197.95
Eggs/gram	186				130
Eggs*1000	40.8				25.7
Eggs after 1 day *1000	28.8				11.5
Temperature (°C)	2.4	6.1	10.3		2.4
Sample size (number of eggs *100	9.6	9.6	9.6		3.8
Hatching date	24-Apr	3-Apr	26-Mar		4-May
Start of metamorphosis	6-Jun	19-May	7-May	19-Apr *	14-Jun
Start of growth experiment	4-Jul	3-Jul	19-Jun	29-May *	19-Jul
Number of fish at start of growth experiment	4	44	65	33 *	34
Code	P1403 2-10	P1403 6-10	P1403 10-10	P1403 10-14	P2703 2-10

TABLE 2

Estimates of growth of 0-group plaice at 15 and 20ºC and at room temperature, at different temperature conditions during egg and larval stage. Fishes that died have not been included in the estimates of growth rates. Growth rates at 15 and 20°C were combined for fish from the same egg incubation conditions.

Ferti-lization date	Temperature (°C) Incubation Eggs	Larvae	Growth Fishes	**Growth rate** (mm/day) mean	maxi-mal	per mm start length	temp. combined	number of dead fishes	of fishes	mean start length
14-Mar	10.3	14.3	15.1	0.85	1.00	0.043 ± 0.008		0	10	20.3
14-Mar	10.3	14.3	19.9	0.96	1.04	0.041 ± 0.003		4	10	20.2
14-Mar	10.3	14.3	15.9	0.62	0.93	0.033 ± 0.008		2	13	17.9
14-Mar	10.3	10.1	15.1	0.68	0.94	0.038 ± 0.015	0.041 ± 0.012	0	10	17.8
14-Mar	10.3	10.1	20.2	0.84	1.07	0.038 ± 0.005	0.039 ± 0.004	4	10	18.2
14-Mar	10.3	10.1	18.0	0.64	0.89	0.033 ± 0.009		7	20	16.6
14-Mar	6.1	10.1	15.2	0.78	0.96	0.039 ± 0.006	0.039 ± 0.006	0	10	20.9
14-Mar	6.1	10.1	20.1	0.74	1.00	0.036 ± 0.003	0.036 ± 0.003	0	10	21.0
14-Mar	6.1	10.1	19.4	0.53	0.86	0.027 ± 0.005		5	24	17.4
14-Mar	2.4	10.1	15.2	0.82	0.93	0.034 ± 0.003		0	4	24.0
27-Mar	2.4	10.1	15	0.52	0.96	0.029 ± 0.010	0.030 ± 0.009	0	10	18.0
27-Mar	2.4	10.1	19.9	0.74	0.93	0.038 ± 0.010	0.038 ± 0.010	2	10	17.5
27-Mar	2.4	10.1	20.1	0.39	0.50	0.027 ± 0.011		10	14	12.6

TABLE 3

Mean values of examined meristic characters in plaice *(Pleuronectes platessa)*, after different incubation conditions during egg and larval stage.

Fertilization date	Inc. Temp.	Larvae Temp.	Vertebrae	Fin rays Dorsal	Fin rays Anal	n
parents		male	43	70	54	
		female	43	76	55	
14-Mar	10.3	14.3	43.36 ± 0.68	69.59 ± 1.85	53.14 ± 1.56	28
14-Mar	10.3	10.1	43.53 ± 0.69	68.54 ± 2.89	51.70 ± 1.61	55
14-Mar	6.1	10.1	42.90 ± 0.45	69.46 ± 2.66	52.51 ± 1.48	39
14-Mar	2.4	10.1	42.50 ± 1.00	69.00 ± 2.16	52.00 ± 1.41	4
27-Mar	2.4	10.1	43.18 ± 0.80	69.27 ± 3.09	51.86 ± 2.32	22
	10.3		43.47 ± 0.69			81
	6.1		42.90 ± 0.45	69.46 ± 2.66	52.51 ± 1.48	39
	2.4		43.08 ± 0.84	69.23 ± 2.93	51.88 ± 2.18	26

TABLE 4
Estimates of egg production of plaice, flounder and dab at different constant temperatures. See also table 5 and 6.

Species	**Plaice**		**Flounder**			**Dab**	
Temperature	3.3 ± 1.10	2.1 ± 0.27	6.0 ± 0.26	10.0 ± 0.32	2.20 ± 0.77	6.0 ± 0.28	10.0 ± 0.31
Gram eggs/fish	328 ± 195	610 ± 218	481 ± 167	246 ± 184	63 ± 66	24 ± 19	6 ± 4
Eggs/fish*1000	60.2 ± 33.0	815.0 ± 290.0	549.0 ± 209.0	269.0 ± 160.0	92.2 ± 118.7	32.4 ± 26.8	10.6 ± 1.0
# Batches	3.3 ± 1.6	6.8 ± 2.8	3.5 ± 1.4	2.3 ± 1.4	4.0 ± 3.2	3.1 ± 2.2	2.0 ± 1.0
Batch size (gram)	103.8 ± 46.7	84.3 ± 48.5	136.1 ± 60.3	113.3 ± 82.2	15.6 ± 13.6	7.5 ± 6.7	5.7 ± 4.0
Batch size (eggs)*1000	19.1 ± 6.9	102.0 ± 48.0	158.0 ± 41.0	155.0 ± 139.0	24.2 ± 22.7	10.2 ± 11.3	8.7 ± 2.5
Spawning period	34.0 ± 18.6	60.9 ± 604.0	19.8 ± 12.2	14.4 ± 5.7	44.3 ± 57.7	46.7 ± 45.5	24.0 ± 21.7
Eggs/gram	187 ± 30	1311 ± 308	1131 ± 299	987 ± 464	1453 ± 451	1215 ± 441	1256 ± 465
n	6	7	11	5	9	15	3

TABLE 5

Egg production of flounders *(Platichthys flesus)* at 3 different temperatures. Parameters marked with an * were not corrected for fishes that died during the experiment. In all other cases, data from fish producing less than 2 batches have not been included.

Temperature (°C)		**2.1** ± 0.27	**6.0** ± 0.26	**10.0** ± 0.32
n (fishes) total	*	10	12	9
Fishes used after correction (n)		7	11	5
Weight fishes average		634.6 ± 129.7	512.6 ± 70.0	651.1 ± 148.5
Number of batches		48	39	12
Minimal number of batches		3	2	1
Maximal number of batches		12	6	5
Batches/fish		6.9 ± 2.8	3.5 ± 1.4	2.3 ± 1.4
Gram eggs/batch	*	84.3 ± 48.5	136.1 ± 60.3	113.3 ± 82.2
Gram eggs total/fish		609.5 ± 217.9	481.1 ± 167.3	245.5 ± 184.4
Gram eggs total/gram fish		0.99 ± 0.39	0.93 ± 0.29	0.39 ± 0.29
Eggs/batch *1000	*	102 ± 48	158 ± 41	155 ± 139
Eggs total/fish *1000		815 ± 290	549 ± 209	269 ± 160
Eggs total/gram fish		1344 ± 604	1066 ± 366	416 ± 261
Spawing period per fish (days)		60.9 ± 28.1	19.8 ± 12.2	14.4 ± 5.7
Spawning period population	*	112	65	48

TABLE 6

Egg production in dab *(Limanda limanda)* at 3 different temperatures. Parameters marked with an * have not been corrected for fish that died during the experiment. In all other cases, data from fish that did not produce eggs have been excluded.

Temperature (°C)		**2.2** ± 0.77	**6.0** ± 0.28	**10.0** ± 0.31
n (fishes) total	*	21	20	21
Fishes used after correction (n)		9	15	3
Weight fishes average		233.6 ± 95.4	248.8 ± 81.9	224.2 ± 26.2
Number of batches		48	47	6
Minimal number of batches		1	1	1
Maximal number of batches		9	8	3
Batches/fish		4.0 ± 3.2	3.1 ± 2.2	2.0 ± 1.0
Gram eggs/batch	*	15.6 ± 13.6	7.5 ± 6.7	5.7 ± 4.0
Gram eggs total/fish		63.2 ± 66.0	23.5 ± 19.0	6.1 ± 3.6
Gram eggs total/gram fish		0.28 ± 0.27	0.09 ± 0.06	0.05 ± 0.03
Eggs/batch *1000	*	24.2 ± 22.7	10.2 ± 11.3	8.7 ± 2.5
Eggs total/fish *1000		92.2 ± 118.7	32.4 ± 26.8	10.6 ± 1.0
Eggs total/gram fish		440 ± 453	127 ± 84	47 ± 4
Spawing period per fish (days)		44.3 ± 57.5	46.7 ± 45.5	24.0 ± 21.7
Spawning period population	*	185	135	17

TABLE 7

Flounder *(Platichthys flesus)*. Days to hatch and first-feeding stage at different constant temperatures.

Hatch		First-feeding	
Temperature (°C)	days D (24 h)	Temperature (°C)	days D (24 h)
2.31 ± 0.08	18.67 ± 1.80	2.32 ± 0.04	33.67 ± 1.37
6.20 ± 0.11	9.88 ± 0.35	6.10 ± 0.00	17.43 ± 0.98
10.31 ± 0.15	6.36 ± 0.81	10.18 ± 0.04	10.11 ± 0.60

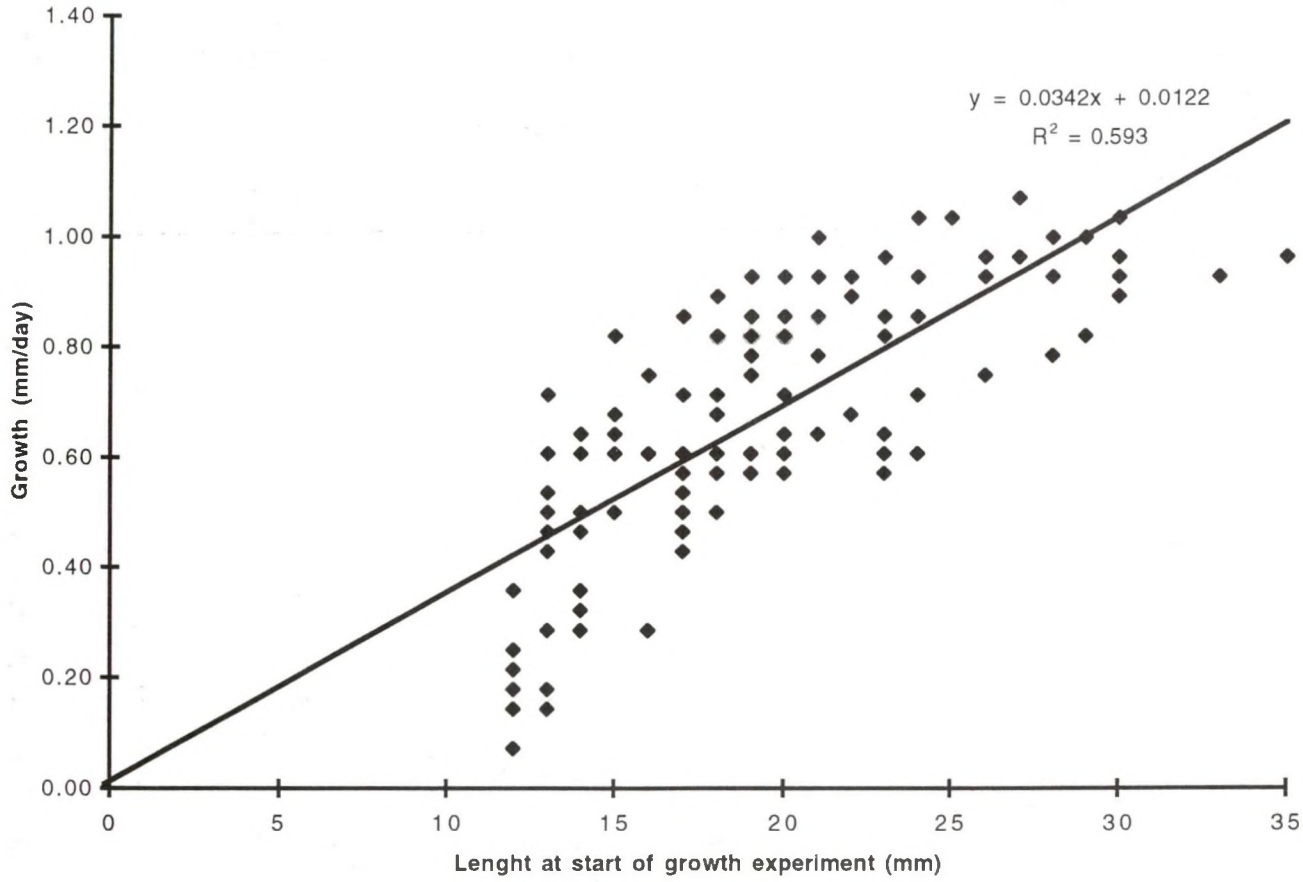

Fig. 1. The relation between length at the start of the growth experiment and the daily growth ($mm.d^{-1}$) of 0-group plaice (*Pleuronectes platessa*). Data from all batches (n=117). Growth ($mm.d^{-1}$)=0.0342*Start-length +0.012. R^2=0.593.

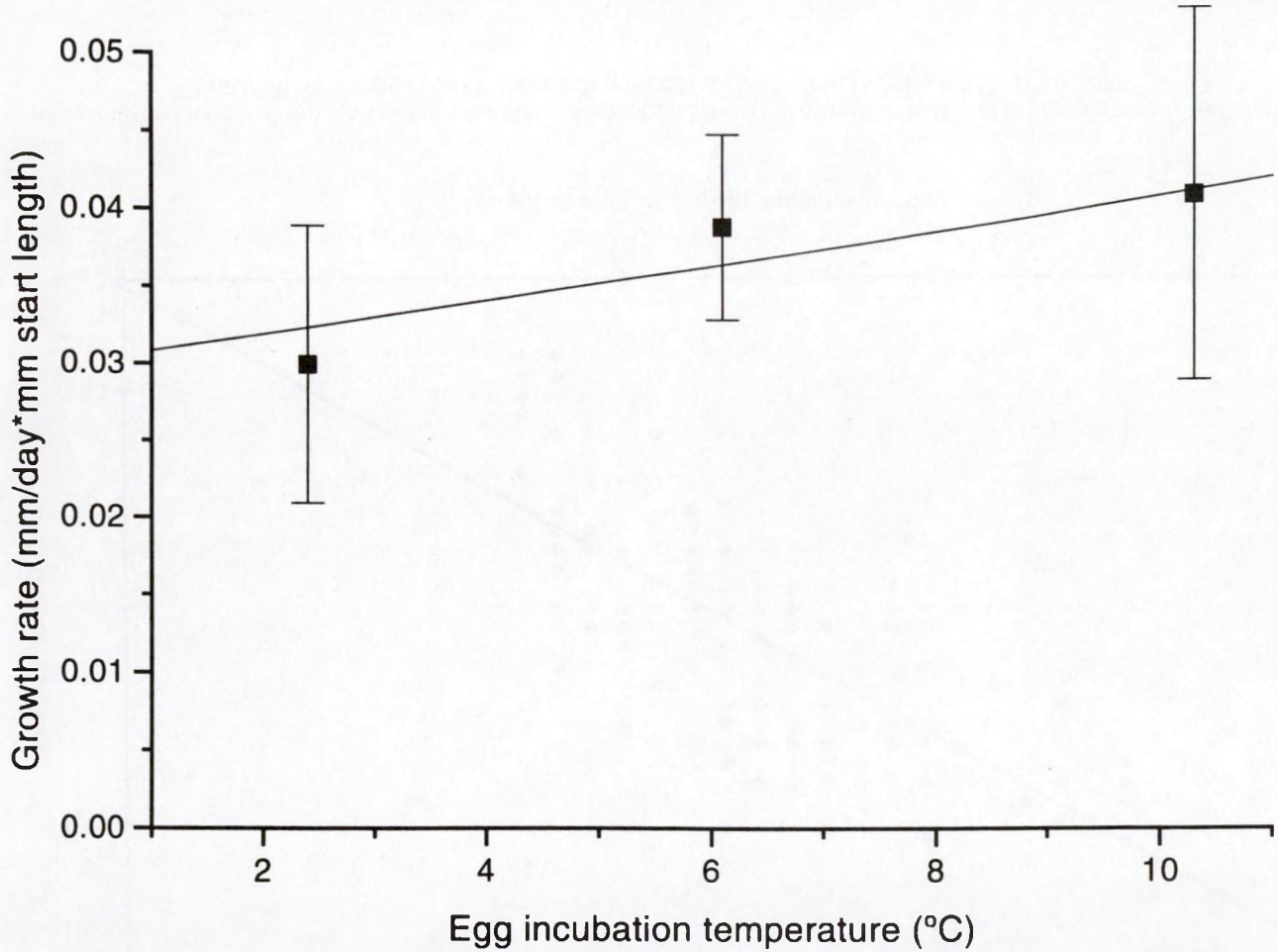

Fig. 2. Growth rates at 15°C of 0-group plaice (*Pleuronectes platessa*) after metamophosis. Comparison of 3 subsamples incubated at 3 different temperatures during egg incubation and early larval development, but reared as larvae at one common temperature of 10ºC until metamophosis.

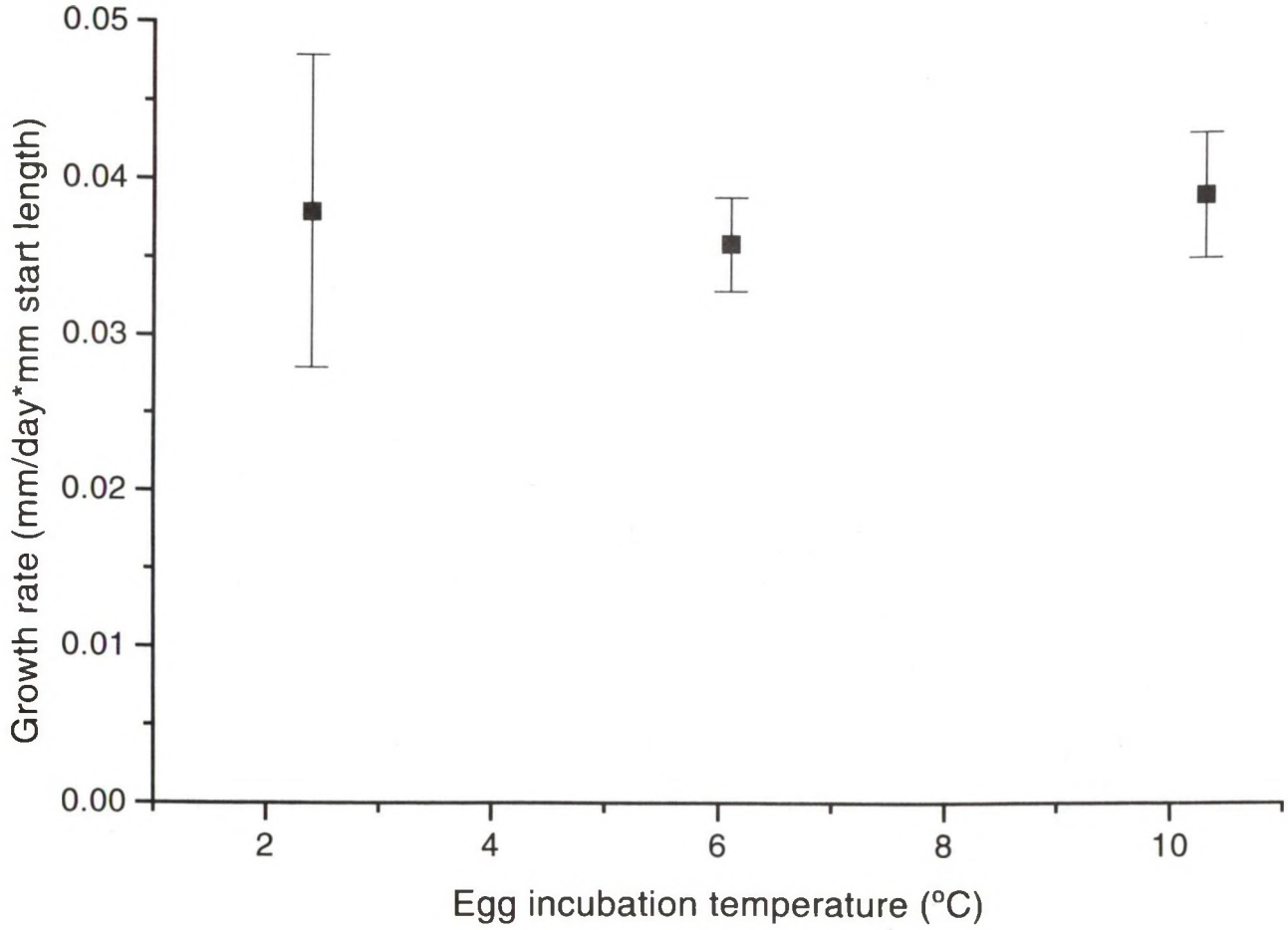

Fig. 3. Growth rate at 20°C of 0-group plaice (*Pleuronectes platessa*) after metamophosis. Comparison of 3 subsamples incubated at 3 different temperatures during egg incubation and early larval development, but reared as larvae at one common temperature of 10ºC until metamophosis.

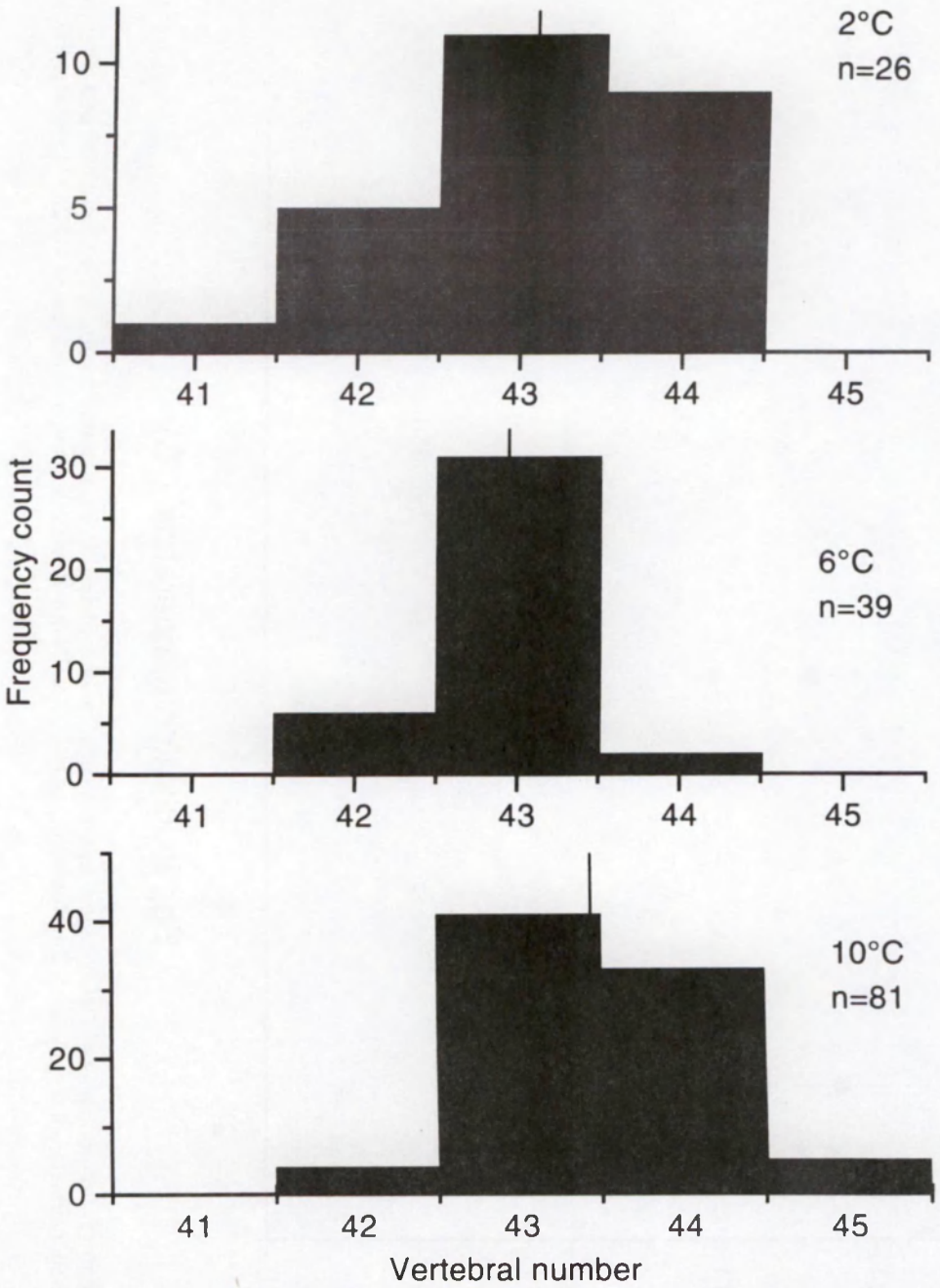

Fig. 4. Number of vertebrae after incubation of juvenile plaice (*Pleuronectes platessa*). Data from subsamples incubated at 2.4, 6.1 and 10.3°C during egg development and early larval development, but reared as larvae at one common temperature of 10°C until metamorphosis. The vertical line indicates the mean number of vertebrae.

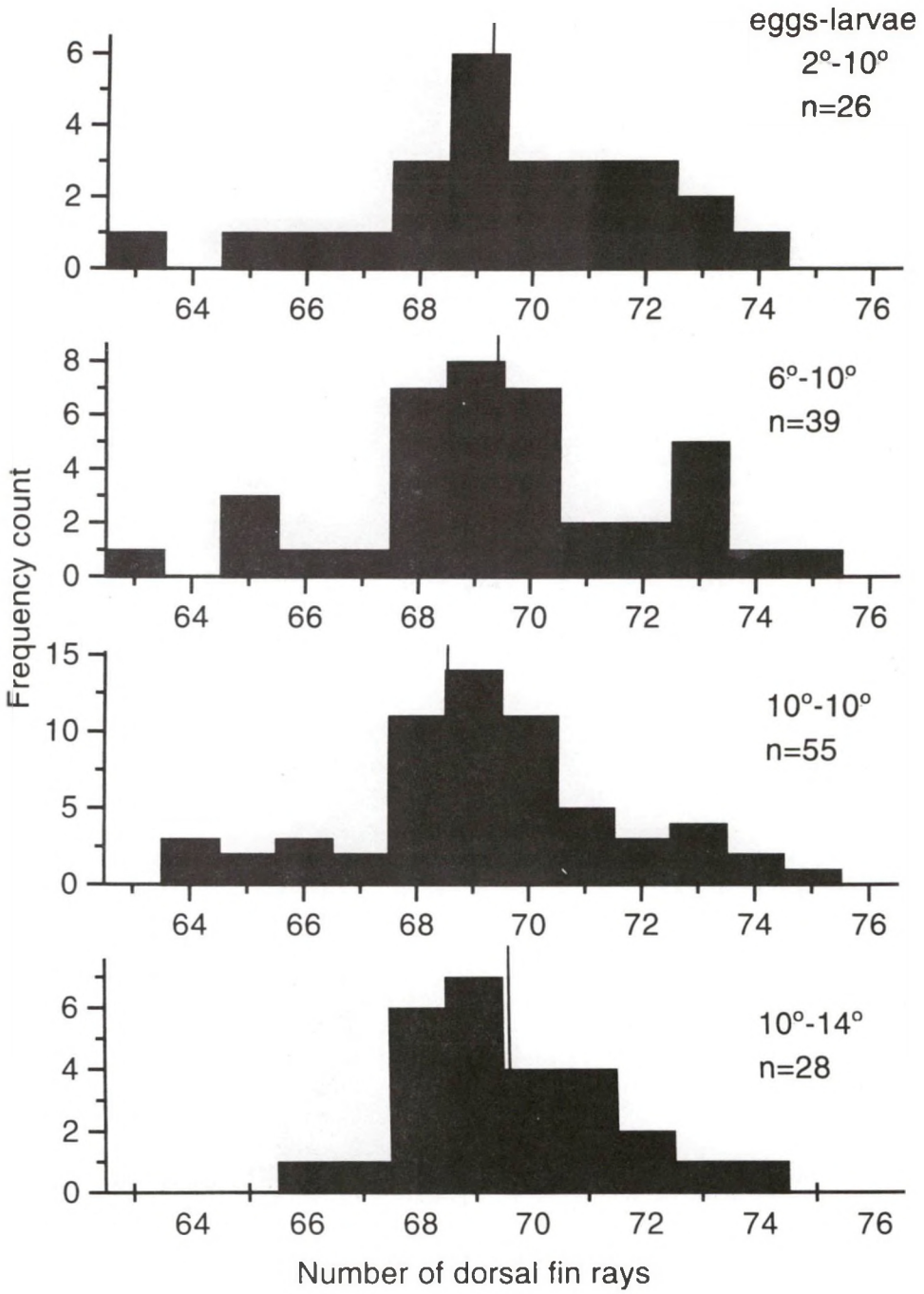

Fig. 5. Number of dorsal fin rays of juvenile plaice (*Pleuronectes platessa)* raised from eggs and larvae incubated at different temperatures. The temperatures indicated are, respectively, the incubation temperature of the eggs and the rearing temperature of the larvae until metamorphosed. The vertical line indicates the mean number of fin rays.

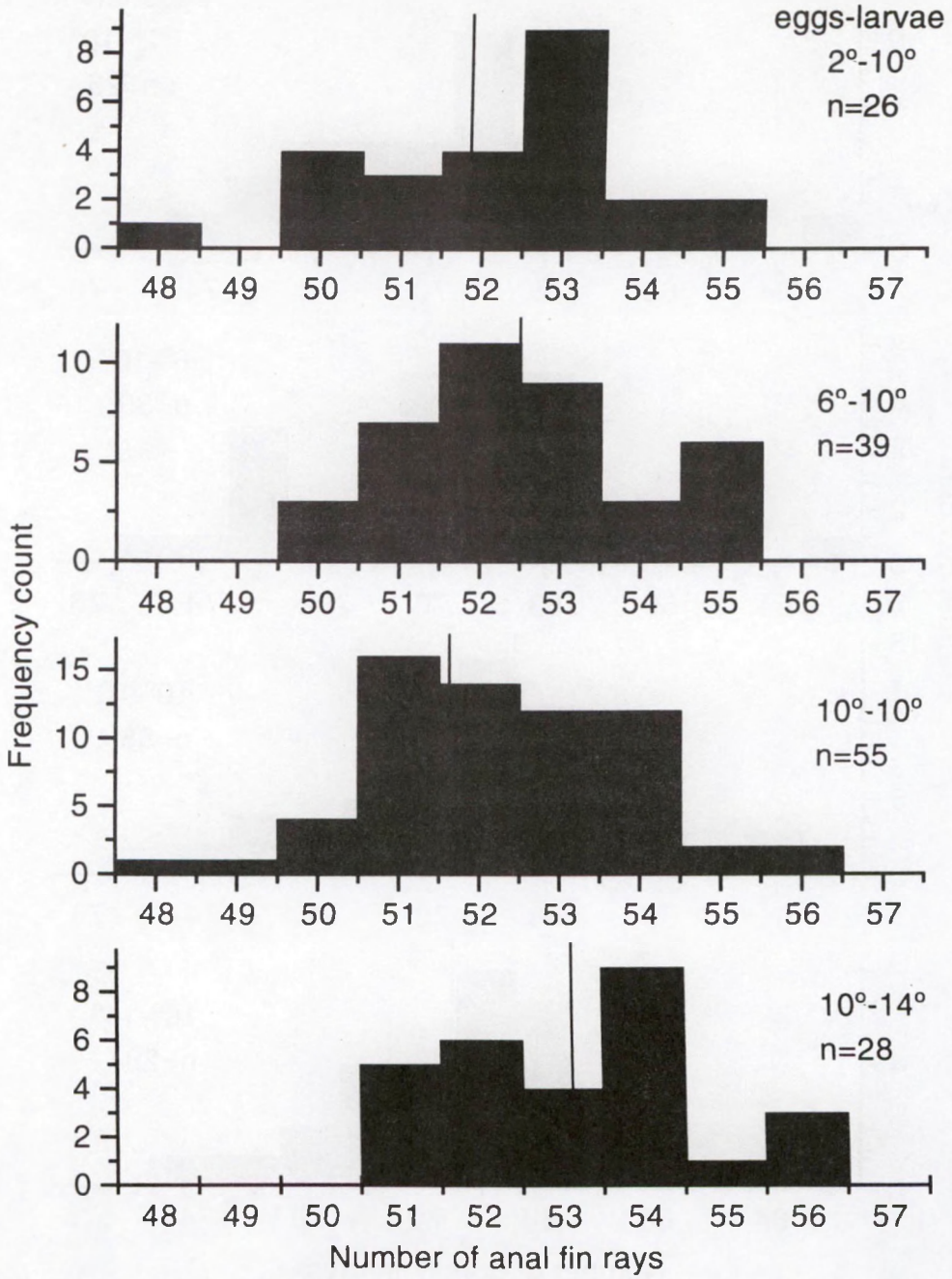

Fig. 6. Number of anal fin rays of juvenile plaice (*Pleuronectes platessa)* raised from eggs and larvae incubated at different temperatures. The temperatures indicated are, respectively, the incubation temperature of the eggs and the rearing temperature of the larvae until metamorphosed. The vertical line indicates the mean number of fin rays.

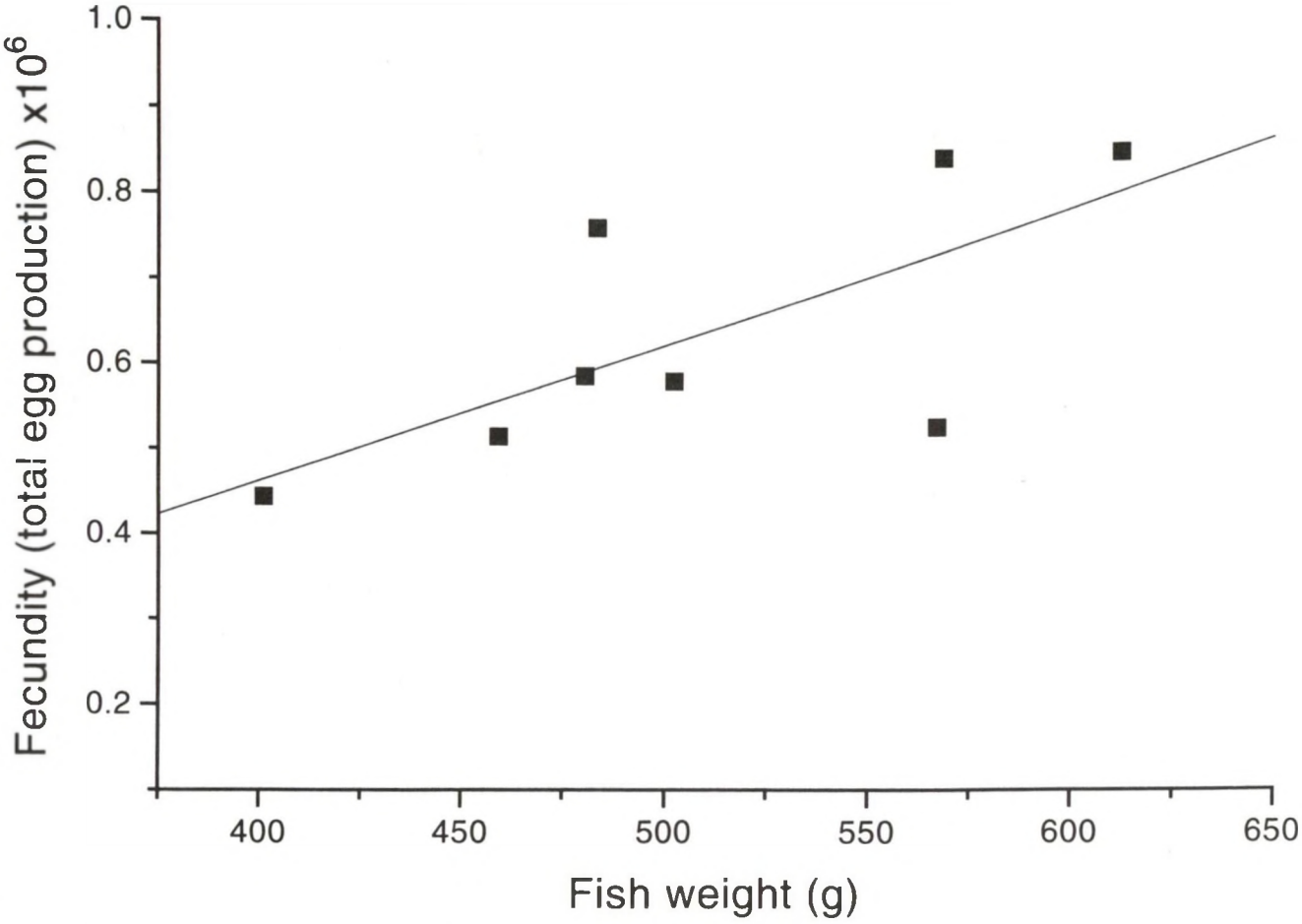

Fig. 7. Total egg production of female flounder (*Platichthys flesus*) kept at 6°C in one spawning season in relation to body weight. Data from animals that died are ommitted. R^2=0.70

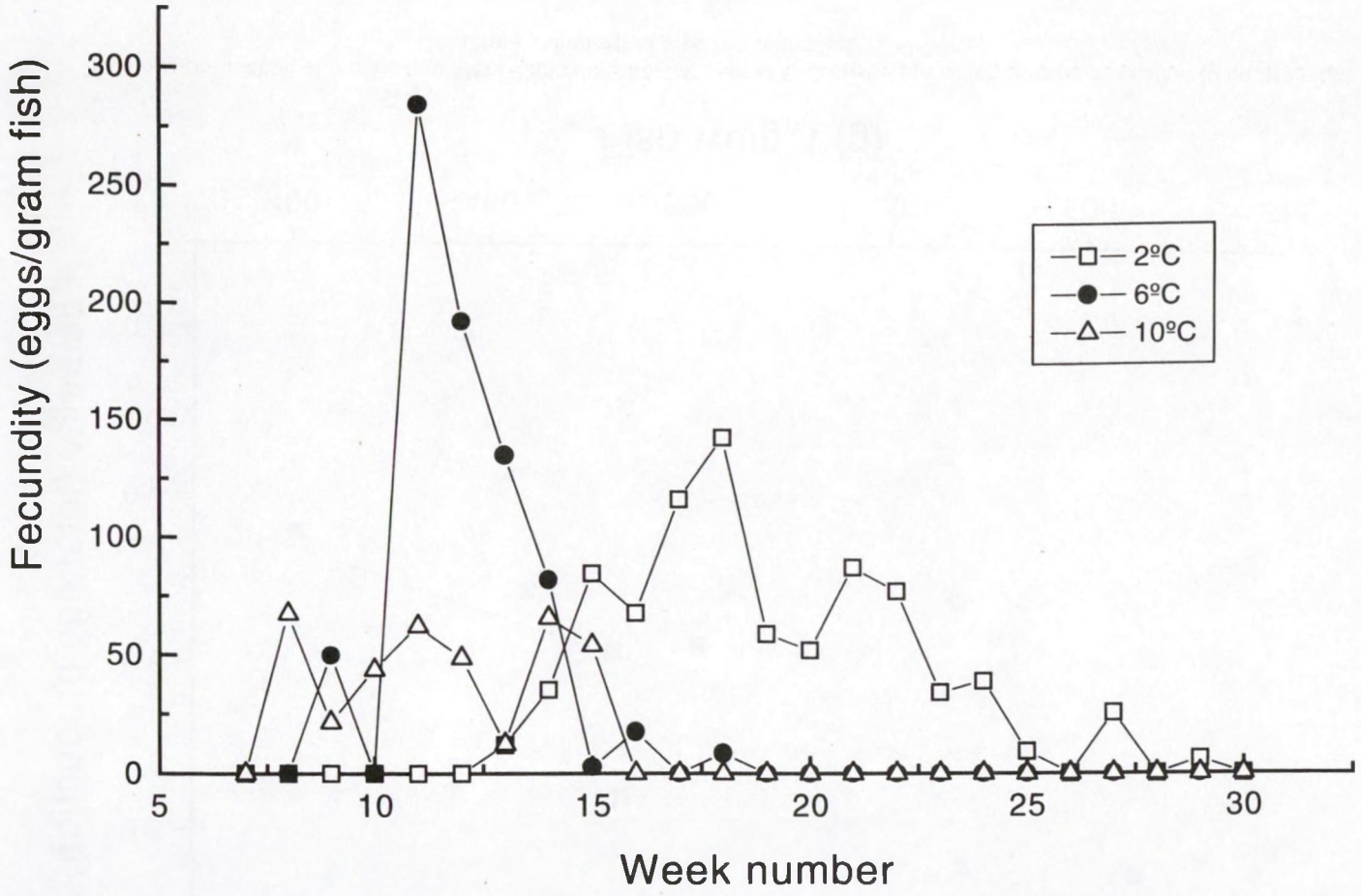

Fig. 8. Total egg production per gram fish per week of female flounder (*Platichthys flesus*) at constant temperatures of 2, 6 and 10°C. Fish weight is the total weight of all fishes in one tank, after they produced their first batch of eggs.

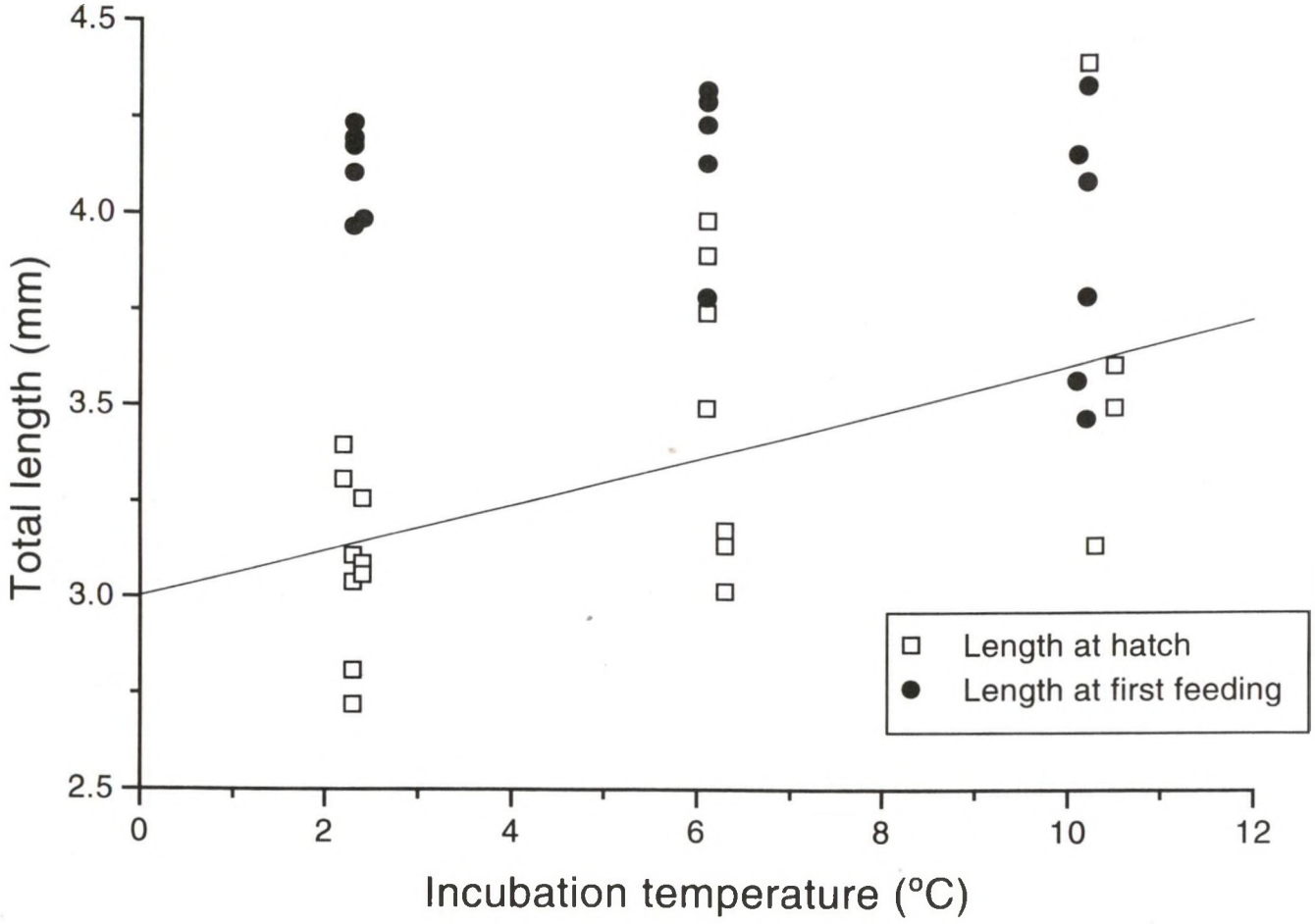

Fig. 9. Length of flounder larvae (*Platichthys flesus*) at two stages of development, at different incubation temperatures. R^2=0.48.

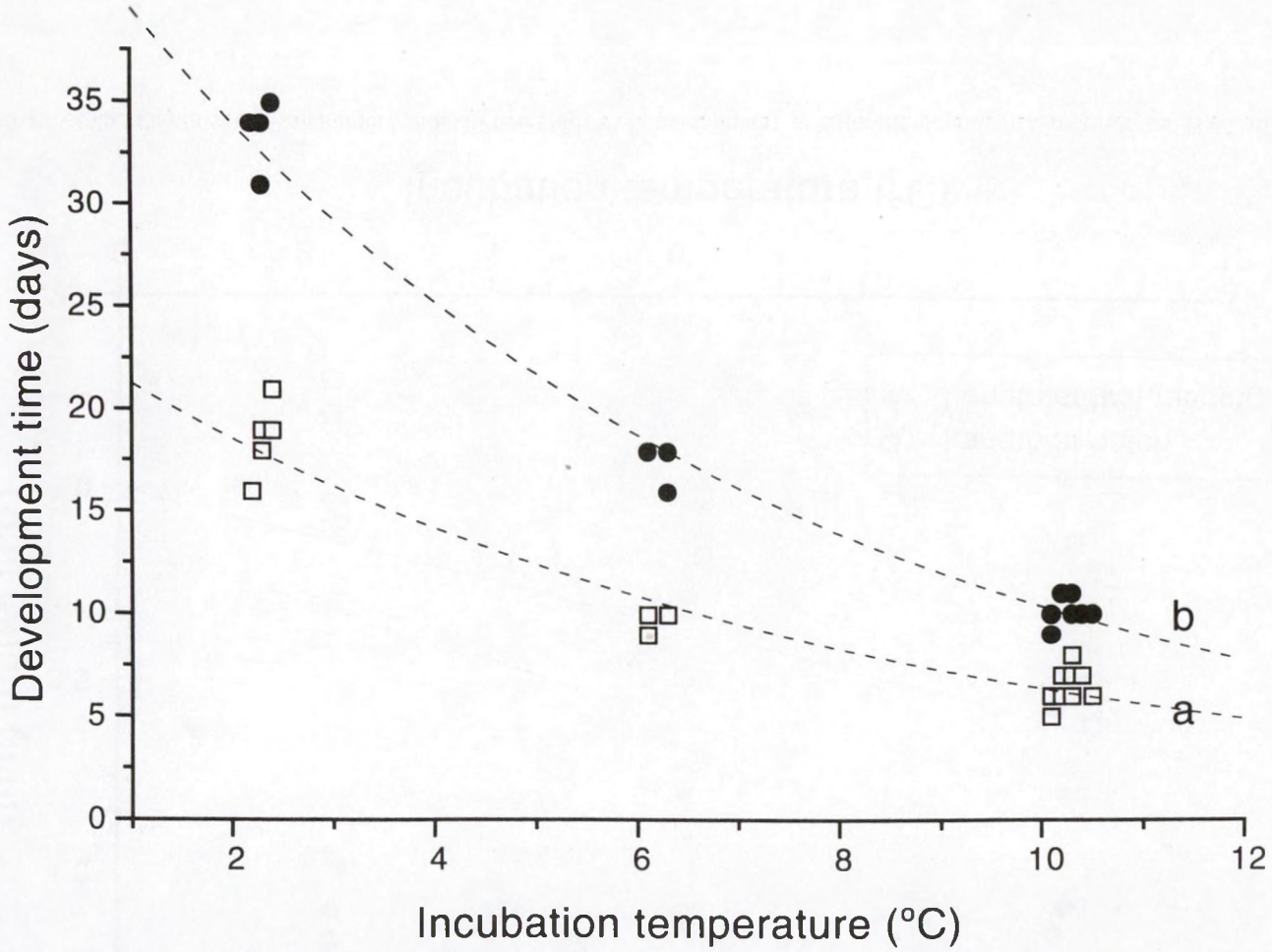

Fig. 10. Incubation time in days of flounder larvae (*Platichthys flesus*) of two stages of embryonic development in relation to incubation temperature. (a) hatch $D=24.3e^{-0.1335T}$, $R^2=0.942$ (b) first feeding $D=45.8e^{-0.1483T}$, $R^2=0.984$.

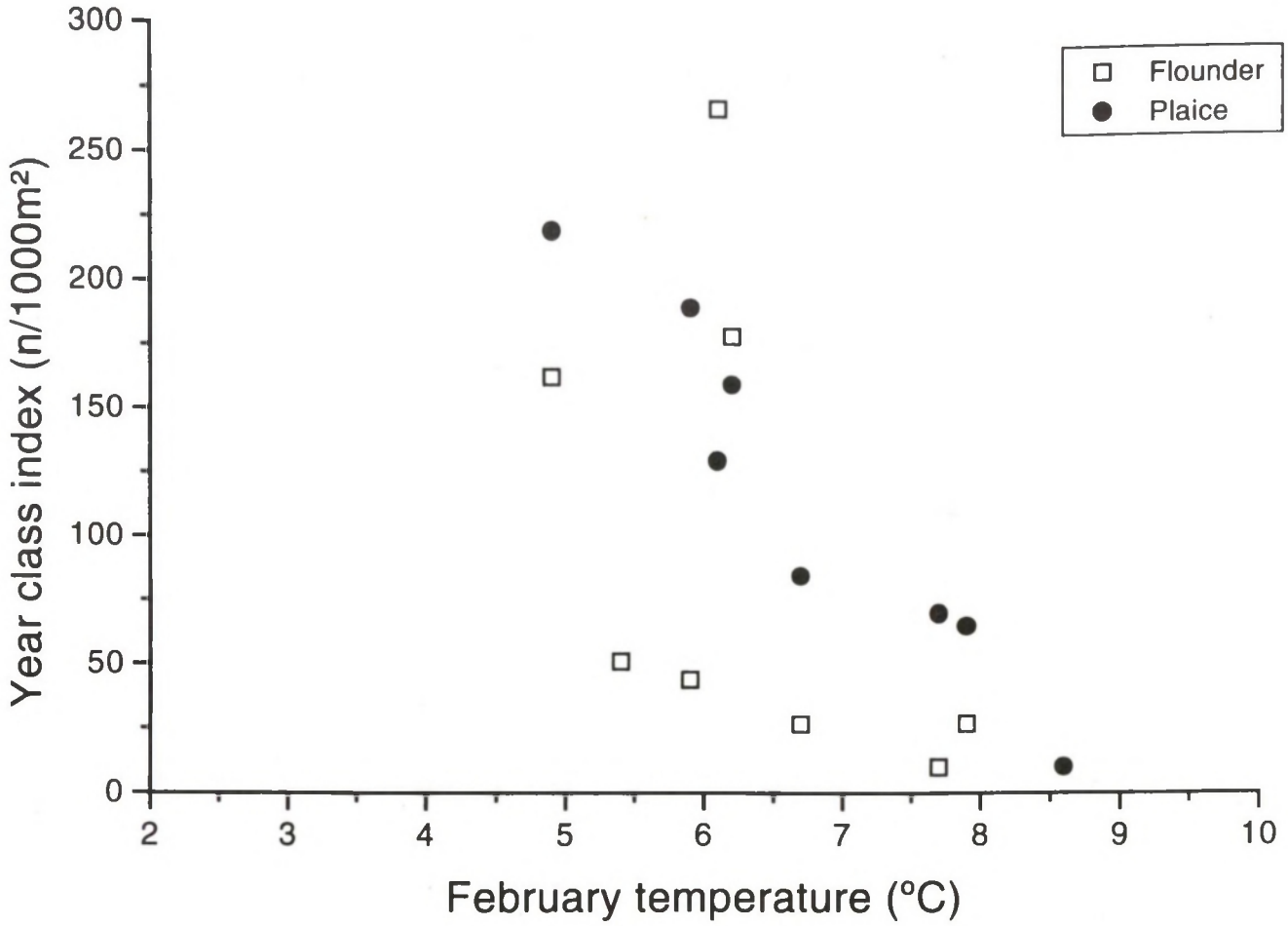

Fig. 11. Relation between water temperature in February on the spawing grounds (from Noordhinder Lightvessel) and year-class strength index of the 0-group of plaice (*Pleuronectes platessa)* and flounder (*Platichthys flesus*) on the Balgzand. Data from VAN DER VEER (1986) and VAN DER VEER *et al.* (1991).

APPENDICES

APPENDIX 1
Staining of juvenile plaice

A method for staining of vertebrae and fin rays of preserved plaice, modified from TÄNING (1944) by SEIGLE & JONES (1995).

1. **Preservation of Samples**. Samples were fixed in a 4% formalin solution for a period of at least 24 h.

2. **Alizarin Stock Solution**. A stock solution of Alizarin is obtained in the as follows.
   a) Dilute 0.5 g of Chloral hydrate in 50 ml. of distilled water.
   b) Dissolve 1 g of alizarin within the mix (is not completely soluble).
   c) Add 5 ml. of acetic acid (glacial) plus 10 ml. of glycerin.
   d) Store in a dark bottle and keep cool.

3. **Stock solution of NaCl**. Stock solution of 1% NaCl in distilled water 5 l of water plus 50 g of NaCl.

4. **Rinsing of Fish**. Fish must be rinsed in a 1% NaCl solution several times to remove all formalin. The salt will help to prevent swelling of the fish due to osmosis. For small fish (10-45 mm), rinsing was completed after 2-4 times over the course of one day. Other studies recommended rinsing at least 3 times every 12 hours. This will vary depending upon the size of the fish.

5. **Bleaching**. This is the most crucial step in the protocol but also one that will vary depending upon the species of the fish, the size of the fish and the thickness of the tissue covering the bones. The dilution's of peroxide can be varied as well as the time spent by each fish in the bleaching solution. For this experiment the following bleaching procedure was used.
   a) For small flatfish (up to 20 mm) bleaching was executed in a 35% peroxide bath for each fish. A few millimeters will suffice such that the fish is completely covered. The time for bleaching in this size range was about 1 h and 20 min.
   b) For larger flatfish (beyond 20 mm) the amount of time spent the bleaching solution was increased so as to allow further penetration of peroxide into the tissue.

   ** It is important to note the color of the fish during the bleaching process. The fish are sufficiently bleached when as they take on a pale yellowish or brown color.

6. **More Rinsing**. After completion of bleaching the fish should are rinsed in the 1% NaCl solution. For the purposes of this experiment, fish were rinsed at least 2 times for 1 h to remove peroxide.
   ** The entire process from steps 2-6 should take about 1 day. Samples can be stored overnight in a solution of NaCl plus 0.5 KOH. (Example: 1 L distilled water + 10 g NaCl + 5 g KOH).

7. **Coloring**.
   a) 2 ml of Alizarin stock solution plus 2 L of 1% NaCl plus 20 g KOH
      ** This wasscaled up or down according to sample size.
   b) Add the fish to stain for 12-48 h regularly checking the status of coloring. Bones will become purple in tint.

8. **Final**. This was done with the same 0.5% KOH+NaCl solution as in step 6.
   a) Change several times as needed.
   b) Finally, store the fish in solution of 50% (KOH+NaCl) and 50% glycerin.

APPENDIX 2

Meristic characters and growth rate of juvenile plaice (*Pleuronectes platessa*) in relation to egg incubation and rearing temperature.

Spawn date	Temperature (°C) Incubation Eggs	Rearing Larvae	Rearing Growth	Fin rays Dorsal	Fin rays Anal	Vertebrae	Growth experiment Length start	Length end	(days)	Growth mm/day	Growth/ mm start length
27-Mar	2.4	10.1	15	71	51	44	12	16	28	0.14	0.012
27-Mar	2.4	10.1	15	74	53	44	12	17	28	0.18	0.015
27-Mar	2.4	10.1	15	63	48	41	12	22	28	0.36	0.030
27-Mar	2.4	10.1	15	69	52	44	13	28	28	0.54	0.041
27-Mar	2.4	10.1	15	69	53	44	15	33	28	0.64	0.043
27-Mar	2.4	10.1	15	69	53	43	18	35	28	0.61	0.034
27-Mar	2.4	10.1	15	72	54	43	20	36	28	0.57	0.029
27-Mar	2.4	10.1	15	70	55	43	20	37	28	0.61	0.030
27-Mar	2.4	10.1	15	71	52	43	23	39	28	0.57	0.025
27-Mar	2.4	10.1	15	68	51	44	35	62	28	0.96	0.028
14-Mar	2.4	10.1	15.2	68	50	44	22	41	28	0.68	0.031
14-Mar	2.4	10.1	15.2	72	53	42	23	46	28	0.82	0.036
14-Mar	2.4	10.1	15.2	67	53	42	23	47	28	0.86	0.037
14-Mar	2.4	10.1	15.2	69	52	42	28	54	28	0.93	0.033
27-Mar	2.4	10.1	19.9				11	dead	28		
27-Mar	2.4	10.1	19.9				11	dead	28		
27-Mar	2.4	10.1	19.9	65	50	42	12	18	28	0.21	0.018
27-Mar	2.4	10.1	19.9	73	55	43	14	28	28	0.50	0.036
27-Mar	2.4	10.1	19.9	62	45	44	15	34	28	0.68	0.045
27-Mar	2.4	10.1	19.9	68	50	43	19	45	28	0.93	0.049
27-Mar	2.4	10.1	19.9	69	53	43	21	45	28	0.86	0.041
27-Mar	2.4	10.1	19.9	70	53	44	22	47	28	0.89	0.041
27-Mar	2.4	10.1	19.9	72	52	43	22	48	28	0.93	0.042
27-Mar	2.4	10.1	19.9	73	53	44	28	54	28	0.93	0.033
27-Mar	2.4	10.1	20.1				11	dead	28		
27-Mar	2.4	10.1	20.1				11	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1				12	dead	28		
27-Mar	2.4	10.1	20.1	71	54	42	13	17	28	0.14	0.011
27-Mar	2.4	10.1	20.1	70	53	43	13	26	28	0.46	0.036
27-Mar	2.4	10.1	20.1	66	50	43	15	29	28	0.50	0.033
27-Mar	2.4	10.1	20.1	69	51	43	17	30	28	0.46	0.027
14-Mar	6.1	10.1	15.2	70	52	44	13	30	28	0.61	0.047
14-Mar	6.1	10.1	15.2	69	53	43	14	31	28	0.61	0.043
14-Mar	6.1	10.1	15.2	71	52	43	15	32	28	0.61	0.040
14-Mar	6.1	10.1	15.2	69	53	42	17	33	28	0.57	0.034
14-Mar	6.1	10.1	15.2	67	51	43	17	37	28	0.71	0.042
14-Mar	6.1	10.1	15.2	68	51	43	20	46	28	0.93	0.046
14-Mar	6.1	10.1	15.2	70	54	43	24	50	28	0.93	0.039
14-Mar	6.1	10.1	15.2	73	53	43	26	53	28	0.96	0.037
14-Mar	6.1	10.1	15.2	69	52	43	30	57	28	0.96	0.032
14-Mar	6.1	10.1	15.2	72	55	43	33	59	28	0.93	0.028
14-Mar	6.1	10.1	19.4				10	dead	28		
14-Mar	6.1	10.1	19.4				12	dead	28		
14-Mar	6.1	10.1	19.4				12	dead	28		
14-Mar	6.1	10.1	19.4				12	dead	28		
14-Mar	6.1	10.1	19.4				13	dead	28		
14-Mar	6.1	10.1	19.4	68	51	42	13	21	28	0.29	0.022
14-Mar	6.1	10.1	19.4	69	52	43	13	21	28	0.29	0.022
14-Mar	6.1	10.1	19.4	65	50	43	14	22	28	0.29	0.020
14-Mar	6.1	10.1	19.4	69	52	43	14	24	28	0.36	0.026
14-Mar	6.1	10.1	19.4	70	53	43	16	24	28	0.29	0.018
14-Mar	6.1	10.1	19.4	68	55	43	17	29	28	0.43	0.025

APPENDIX 2 CONTINUED

Spawn date	Temperature (°C)			Fin rays		Vertebrae	Growth experiment				Growth/
	Incubation	Rearing		Dorsal	Anal		Length		(days)	Growth	mm start
	Eggs	Larvae	Growth				start	end		mm/day	length
14-Mar	6.1	10.1	19.4	70	52	42	17	31	28	0.50	0.029
14-Mar	6.1	10.1	19.4	68	52	43	18	32	28	0.50	0.028
14-Mar	6.1	10.1	19.4	74	55	43	18	34	28	0.57	0.032
14-Mar	6.1	10.1	19.4	70	52	43	18	35	28	0.61	0.034
14-Mar	6.1	10.1	19.4	73	55	43	19	35	28	0.57	0.030
14-Mar	6.1	10.1	19.4	70	52	43	19	36	28	0.61	0.032
14-Mar	6.1	10.1	19.4	72	53	42	20	36	28	0.57	0.029
14-Mar	6.1	10.1	19.4	71	54	43	21	39	28	0.64	0.031
14-Mar	6.1	10.1	19.4	73	55	43	23	40	28	0.61	0.026
14-Mar	6.1	10.1	19.4	69	53	43	24	41	28	0.61	0.025
14-Mar	6.1	10.1	19.4	63	50	43	23	41	28	0.64	0.028
14-Mar	6.1	10.1	19.4	70	51	43	24	48	28	0.86	0.036
14-Mar	6.1	10.1	19.4	68	53	43	28	50	28	0.79	0.028
14-Mar	6.1	10.1	20.1	66	51	42	13	27	28	0.50	0.038
14-Mar	6.1	10.1	20.1	65	52	42	14	28	28	0.50	0.036
14-Mar	6.1	10.1	20.1	68	51	43	16	33	28	0.61	0.038
14-Mar	6.1	10.1	20.1	69	52	43	17	34	28	0.61	0.036
14-Mar	6.1	10.1	20.1	69	54	43	18	37	28	0.68	0.038
14-Mar	6.1	10.1	20.1	68	51	44	21	43	28	0.79	0.037
14-Mar	6.1	10.1	20.1	73	53	43	26	47	28	0.75	0.029
14-Mar	6.1	10.1	20.1	65	50	43	27	54	28	0.96	0.036
14-Mar	6.1	10.1	20.1	73	53	43	28	56	28	1.00	0.036
14-Mar	6.1	10.1	20.1	75	55	43	30	57	28	0.96	0.032
14-Mar	10.3	10.1	15.1				12	14	28	0.07	0.006
14-Mar	10.3	10.1	15.1				13	21	28	0.29	0.022
14-Mar	10.3	10.1	15.1	69	51	44	13	33	28	0.71	0.055
14-Mar	10.3	10.1	15.1	74	52	43	16	37	28	0.75	0.047
14-Mar	10.3	10.1	15.1	73	54	44	16	37	28	0.75	0.047
14-Mar	10.3	10.1	15.1	69	52	43	17	41	28	0.86	0.050
14-Mar	10.3	10.1	15.1	68	51	42	18	41	28	0.82	0.046
14-Mar	10.3	10.1	15.1	69	53	44	19	42	28	0.82	0.043
14-Mar	10.3	10.1	15.1	70	51	44	24	44	28	0.71	0.030
14-Mar	10.3	10.1	15.1	75	55	44	30	59	28	1.04	0.035
14-Mar	10.3	10.1	18.0				10	dead	28		
14-Mar	10.3	10.1	18.0				11	dead	28		
14-Mar	10.3	10.1	18.0				11	dead	28		
14-Mar	10.3	10.1	18.0				12	dead	28		
14-Mar	10.3	10.1	18.0				12	dead	28		
14-Mar	10.3	10.1	18.0			44	13	dead	28		
14-Mar	10.3	10.1	18.0		53	45	13	dead	28		
14-Mar	10.3	10.1	18.0	72	56	43	13	18	28	0.18	0.014
14-Mar	10.3	10.1	18.0	69	50	43	14	22	28	0.29	0.020
14-Mar	10.3	10.1	18.0	70	54	43	14	27	28	0.46	0.033
14-Mar	10.3	10.1	18.0	69	53	43	14	32	28	0.64	0.046
14-Mar	10.3	10.1	18.0	68	53	44	16	33	28	0.61	0.038
14-Mar	10.3	10.1	18.0	66	51	43	17	34	28	0.61	0.036
14-Mar	10.3	10.1	18.0	73	54	44	18	38	28	0.71	0.040
14-Mar	10.3	10.1	18.0	69	52	44	18	38	28	0.71	0.040
14-Mar	10.3	10.1	18.0	72	54	44	19	40	28	0.75	0.039
14-Mar	10.3	10.1	18.0	73	52	44	19	41	28	0.79	0.041
14-Mar	10.3	10.1	18.0	70	53	44	29	52	28	0.82	0.028
14-Mar	10.3	10.1	18.0	68	53	44	29	52	28	0.82	0.028
14-Mar	10.3	10.1	18.0	69	51	44	30	55	28	0.89	0.030
14-Mar	10.3	10.1	20.2				12		28		
14-Mar	10.3	10.1	20.2				12		28		
14-Mar	10.3	10.1	20.2				12		28		
14-Mar	10.3	10.1	20.2			43	16	dead	28		
14-Mar	10.3	10.1	20.2	66	51	43	17	32	28	0.54	0.032
14-Mar	10.3	10.1	20.2	72	53	43	18	35	28	0.61	0.034
14-Mar	10.3	10.1	20.2	68	52	44	19	43	28	0.86	0.045
14-Mar	10.3	10.1	20.2	71	52	44	20	44	28	0.86	0.043
14-Mar	10.3	10.1	20.2	70	53	43	27	57	28	1.07	0.040
14-Mar	10.3	10.1	20.2	70	51	43	29	57	28	1.00	0.034

APPENDIX 2 CONTINUED

Spawn date	Temperature (°C) Incubation Eggs	Rearing Larvae	Growth	Fin rays Dorsal	Anal	Vertebrae	Growth experiment Length start	end	(days)	Growth mm/day	Growth/ mm start length
14-Mar	10.3	10.1		61	51	43	10				
14-Mar	10.3	10.1		67	50	42	11				
14-Mar	10.3	10.1		68	52	43	11				
14-Mar	10.3	10.1		64	48	43	11				
14-Mar	10.3	10.1		70	53	44	11				
14-Mar	10.3	10.1		64	52	44	11				
14-Mar	10.3	10.1		64	51	43	11				
14-Mar	10.3	10.1		65	50	45	11				
14-Mar	10.3	10.1		68	50	43	12				
14-Mar	10.3	10.1		69	49	43	12				
14-Mar	10.3	10.1		65	51	43	12				
14-Mar	10.3	10.1		70	51	45	12				
14-Mar	10.3	10.1		70	53	44	12				
14-Mar	10.3	10.1		70	51	43	12				
14-Mar	10.3	10.1		66	51	44	12				
14-Mar	10.3	10.1		69	50	43	12				
14-Mar	10.3	10.1		69	53	43	13				
14-Mar	10.3	10.1		69	50	44	13				
14-Mar	10.3	10.1		70	51	43	13				
14-Mar	10.3	10.1		67	52	44	13				
14-Mar	10.3	10.1		64	52	45	13				
14-Mar	10.3	10.1		64	48	43	15				
14-Mar	10.3	10.1		65	50	43	16				
14-Mar	10.3	10.1		68	51	44	16				
14-Mar	10.3	10.1		66	50	43	17				
14-Mar	10.3	14.3	15.1	69	53	43	13	25	28	0.43	0.033
14-Mar	10.3	14.3	15.1	70	54	44	15	38	28	0.82	0.055
14-Mar	10.3	14.3	15.1	72	56	44	17	41	28	0.86	0.050
14-Mar	10.3	14.3	15.1	72	53	43	18	43	28	0.89	0.050
14-Mar	10.3	14.3	15.1	68	51	44	19	43	28	0.86	0.045
14-Mar	10.3	14.3	15.1	66	51	43	21	47	28	0.93	0.044
14-Mar	10.3	14.3	15.1	70	56	43	21	49	28	1.00	0.048
14-Mar	10.3	14.3	15.1	68	53	43	23	50	28	0.96	0.042
14-Mar	10.3	14.3	15.1	68	54	44	26	52	28	0.93	0.036
14-Mar	10.3	14.3	15.1	73	54	44	30	56	28	0.93	0.031
14-Mar	10.3	14.3	15.9				12	dead	28		
14-Mar	10.3	14.3	15.9	67	51	43	12	19	28	0.25	0.021
14-Mar	10.3	14.3	15.9	68	52	43	14	23	28	0.32	0.023
14-Mar	10.3	14.3	15.9	69	52	45	14	24	28	0.36	0.026
14-Mar	10.3	14.3	15.9	71	54	43	15	29	28	0.50	0.033
14-Mar	10.3	14.3	15.9				16	dead	28		
14-Mar	10.3	14.3	15.9	69	54	43	17	30	28	0.46	0.027
14-Mar	10.3	14.3	15.9	71	55	43	20	38	28	0.64	0.032
14-Mar	10.3	14.3	15.9	69	52	42	20	40	28	0.71	0.036
14-Mar	10.3	14.3	15.9	70	53	44	20	43	28	0.82	0.041
14-Mar	10.3	14.3	15.9	68	51	44	21	47	28	0.93	0.044
14-Mar	10.3	14.3	15.9	69	51	44	22	47	28	0.89	0.041
14-Mar	10.3	14.3	15.9	69	54	43	24	50	28	0.93	0.039
14-Mar	10.3	14.3	19.9				12	dead	28		
14-Mar	10.3	14.3	19.9				15	dead	28		
14-Mar	10.3	14.3	19.9				17	dead	28		
14-Mar	10.3	14.3	19.9		52	43	18	dead	28		
14-Mar	10.3	14.3	19.9	70	56	42	21	45	28	0.86	0.041
14-Mar	10.3	14.3	19.9	69	52	44	21	45	28	0.86	0.041
14-Mar	10.3	14.3	19.9	71	54	43	22	48	28	0.93	0.042
14-Mar	10.3	14.3	19.9	68	54	43	24	53	28	1.04	0.043
14-Mar	10.3	14.3	19.9	71	54	44	25	54	28	1.04	0.041
14-Mar	10.3	14.3	19.9	74	52	43	27	54	28	0.96	0.036

APPENDIX 3

Estimates of the egg production of individual plaice (*Pleuronectes platessa*).

Temp. (°C)	Fish length (cm)	Fish weight (g) before spawning	Fish weight (g) after period	Number of batches	Gram egg per batch	Total weight eggs(g)	Number of eggs per batch	Total number of eggs	Spawning start	Spawning end	Spawning period (days)	Total Eggs (g)/ gram fish	Total Eggs/ gram fish
3.3±1.10	35.5	436.7	436.7	1	90.7	90.7	17573	17573	27-Mar	28-Mar	1	0.21	40
3.3±1.10	36.0	519.5	448.9	3	90.6	271.9	16666	49998	11-Apr	16-May	35	0.52	96
3.3±1.10	36.5	465.0	450.6	2	69.2	138.3	14420	28841	16-May	12-Jun	27	0.30	62
3.3±1.10	37.3	550.0	580.2	5	85.0	424.9	16529	82645	1-Feb	27-Mar	54	0.77	150
3.3±1.10	38.8	681.0	577.7	5	89.6	448.2	16551	82755	1-Feb	14-Mar	41	0.66	122
3.3±1.10	41.0	724.1	678.9	4	197.7	593.0	33127	99380	9-Feb	27-Mar	46	0.82	137

APPENDIX 4

Estimates of the egg production of individual flounder *(Platichtys flesus)*. Fishes marked with an * were not used for determining mean egg production, because of a preliminary death or an extra ordinary low egg production.

Temp. (°C)	Fish length (cm)	Fish weight (g) before spawning	Fish weight (g) after period	Number of batches	Gram egg per batch	Total weight eggs(g)	Number of eggs per batch	Total number of eggs	Spawning start	Spawning end	Spawning period (days)	Way of leaving	Date of death	Preli-minary death	Total Eggs (g)/ gram fish	Total Eggs/ gram fish
2.1±.027	34.5	506.4	391.5	7	97.3	680.9	160700	1124900	3-Apr	8-May	35	left	-		1.34	2221
2.1±.027	35.5	540.8	400.1	12	69.4	833.3	93310	1119717	3-Apr	4-Jul	92	left	-		1.54	2070
2.1±.027	34.5 *	481.1	481.1	1	75.9	75.9	84180	84180	30-Mar	31-Mar	1	died	12-Apr	no	0.16	175
2.1±.027	38.5	699.7	492.6	7	95.6	860.6	140863	986040	13-Apr	20-Jul	98	left	-		1.23	1409
2.1±.027	36.0	617.1	504.0	8	55.6	445.0	79017	632136	19-Apr	8-Jun	50	left	-		0.72	1024
2.1±.027	38.0	762.8	580.0	6	121.0	726.2	151856	911139	19-Apr	4-Jul	76	left	-		0.95	1194
2.1±.027	38.0 *	768.7	750.1	2	59.4	118.7	58616	117232	4-May	11-May	7	died	2-Jun	no	0.15	153
2.1±.027	36.0	494.0	419.0	3	110.1	330.2	146807	440420	4-May	30-May	26	left	-		0.67	892
2.1±.027	36.5 *	510.9	510.9	1	11.2	11.2	10208	10208	9-May	10-May	1	died	9-May	no	0.02	20
2.1±.027	38.5	821.5	603.8	5	78.0	390.1	98472	492360	27-Apr	15-Jun	49	died	22-Jun	no	0.47	599
6.0±0.26	31.5	401.1	362.4	3	117.3	351.9	147955	443866	16-Mar	3-Apr	18	left	-		0.88	1107
6.0±0.26	33.0	459.1	401.4	6	87.9	527.7	85840	515040	13-Mar	4-May	52	died	30-May	yes	1.15	1122
6.0±0.26	33.0	421.5	417.0	2	76.7	153.4	83638	167276	20-Mar	13-Apr	24	left	-		0.36	397
6.0±0.26	34.0	483.3	402.8	5	102.9	514.3	152092	760460	13-Mar	24-Mar	11	died	23-May	yes	1.06	1573
6.0±0.26	34.0	557.5	534.5	2	188.7	377.3	188123	376246	13-Mar	20-Mar	7	left	-		0.68	675
6.0±0.26	34.5	567.1	402.8	3	182.0	545.9	175703	527109	13-Mar	3-Apr	21	left	-		0.96	929
6.0±0.26	35.0	480.3	417.3	4	152.0	608.0	146752	587008	13-Mar	27-Mar	14	died	30-May	yes	1.27	1222
6.0±0.26	35.5	585.2	461.9	2	184.9	369.8	197522	395044	28-Feb	13-Mar	13	left	-		0.63	675
6.0±0.26	35.5	612.4	453.2	4	172.7	690.6	212040	848160	28-Feb	28-Mar	28	died	27-Apr	no	1.13	1385
6.0±0.26	35.5	502.3	451.1	3	141.0	423.1	193717	581151	13-Mar	28-Mar	15	left	-		0.84	1157
6.0±0.26	36.5	568.5	492.1	5	145.9	729.6	168503	842515	13-Mar	28-Mar	15	died	29-May	yes	1.28	1482
6.0±0.26	37.0 *	621.5	621.5	1	153.6	153.6	138240	138240	28-Feb	13-Mar	13	left	-		0.25	222
10.0±0.32	33.0	494.0	399.0	3	88.2	264.7	100382	301146	10-Mar	3-Apr	24	died	10-Apr	yes	0.54	610
10.0±0.32	33.5	529.0	529.0	2	44.7	31.7	23143	23143	10-Mar	20-Mar	10		-		0.06	44
10.0±0.32	34.0 *	436.0	436.0	1	156.0	156.0	405600	405600	21-Feb	22-Feb	1	died	19-Apr	yes	0.36	930
10.0±0.32	37.0	670.0	566.1	5	86.3	518.0	76503	459015	10-Mar	24-Mar	14	died	3-Apr	no	0.77	685
10.0±0.32	37.0 *	574.5	614.2	2	38.8	77.7	34290	68581	20-Mar	21-Mar	1	died	3-Apr	no	0.14	119
10.0±0.32	38.0 *	694.7	694.7	1	179.1	179.1	158461	158461	13-Mar	14-Mar	1	died	4-Apr	no	0.26	228
10.0±0.32	40.0	866.0	719.4	2	63.4	126.9	118038	236075	28-Feb	10-Mar	10	died	27-Mar	no	0.15	273
10.0±0.32	40.5	696.3	696.3	1	286.2	286.2	326135	326135	27-Mar	10-Apr	14	died	18-Apr	no	0.41	468
10.0±0.32	38.0 *	647.5		0		0.0		0			0	died	16-Mar	no	0.00	0

APPENDIX 5

Estimates of the egg production of individual dab *(Limanda limanda)* kept at different temperatures.

Temp. (°C)	Fish length (cm)	Fish weight (g) before spawning	Fish weight (g) after period	Number of batches	Gram eggs per batch	Total weight eggs(g)	Number of eggs per batch	Total number of eggs	Spawning start	Spawning end	Spawnin period (days)	Way of leaving	Date of death	Total Eggs (g)/ gram fish	Eggs/ gram fish
10	21.5		107.3	0											
10	24.5		180.5	0											
10	25.0		144.3	0								left	23-May		
10	25.0		168.4	0								left	23-May		
10	25.5		172.3	0								left	23-May		
10	26.0		163.8	0								left	23-May		
10	26.0		198.8	0								left	23-May		
10	27.5		270.2	0								left	23-May		
10	28.0		239.5	0								left	20-Apr		
10	28.0		226	0								left	20-Apr		
10	28.5		240	0								left	23-May		
10	28.5		279.2	0								left	23-May		
10	29.0		238.8	0								left	23-May		
10	29.0		305	0								left	23-May		
10	30.0		237	0								left	20-Apr		
10	31.0		348.8	0								died	3-May		
10	31.5		315	0								left	20-Apr		
10	31.5		377.5	0								left	23-May		
10	26.0	199.6	199.6	1	6.34	6.34	9510	9510	28-Feb	1-Mar	1	left	8-Jun	0.03	48
10	25.0	221.2	210.2	2	9.54	19.09	5729	11457	17-Mar	13-Apr	27	died	26-Apr	0.09	52
10	25.0	251.8	232	3	2.40	7.20	3600	10800	28-Feb	13-Apr	44	died	5-May	0.03	43
6	22.0		132.5	0								died	10-Jul		
6	25.5		153.1	0								left			
6	27.5		197.6	0								left			
6	27.5		240.7	0								left			
6	29.5		275.5	0								left			
6	23.5	156.8	156.8	1	23.40	23.40	33051	33051	17-Mar	18-Mar	1	died	26-Jul	0.15	211
6	24.5	177.4	177.4	1	4.07	4.07	7432	7432	13-Apr	14-Apr	1	left		0.02	42
6	24.5	179.5	179.5	1	4.48	4.48	7664	7664	2-Jun	3-Jun	1	left		0.02	43
6	26.5	268.2	268.2	1	9.13	9.13	14478	14478	13-Apr	14-Apr	1	left		0.03	54
6	30	333.6	333.6	1	11.47	11.47	17755	17755	20-Apr	21-Apr	1	left		0.03	53
6	24.0	175.0	170.5	2	3.86	7.72	5201	10402	6-Apr	9-Jun	64	left		0.04	59
6	27.0	265.5	244.4	2	15.31	30.62	27175	54350	13-Apr	20-Apr	7	left		0.12	205
6	23.5	163.2	172.2	3	4.38	13.13	3691	11072	20-Apr	9-Jun	50	left		0.08	68
6	26.5	238.9	247.1	3	6.90	20.69	10538	31613	20-Apr	11-May	21	left		0.09	132
6	30	374.5	323.1	3	14.65	43.96	22013	66039	28-Feb	2-Jun	94	left		0.12	176
6	28	276.0	266.8	4	3.98	15.90	4693	18772	2-May	16-Jun	45	left		0.06	68
6	25.0	199.7	167.6	5	6.75	33.74	8312	41559	6-Apr	4-Jul	89	died	31-Jul	0.17	208
6	29.5	336.6	315.9	6	12.82	76.93	17679	106072	13-Mar	13-Jul	122	died	17-Jul	0.23	315
6	30.5	403.4	323.2	6	4.05	24.32	5435	32608	13-Mar	4-Jul	113	left		0.06	81
6	24.5	183.1	159.9	8	4.10	32.83	2598	33425	13-Apr	13-Jul	91	died	13-Jul	0.18	183
2	20.0	74.6		0							0	died	24-Aug	0.000	0
2	24.5	179.2		0		0.0		0			0	died	27-Apr	0.000	0
2	24.5	134.1		0							0			0.000	0
2	24.5	133.2		0							0			0.000	0
2	25	191		0							0			0.000	0
2	25.5	190.2		0							0			0.000	0
2	25.5	148.2		0							0			0.000	0
2	27.0	212.7		0		0.0		0			0	died	2-May	0.000	0
2	30.5	335.4		0		0.0		0			0	died	27-Apr	0.000	0
2	23.0	131.6	131.6	1	12.3	12.3	14277	14277	4-Jul	5-Jul	1			0.094	108
2	24.0	179.6	179.6	1	1.7	1.7	987	987	24-Aug	25-Aug	1			0.010	5
2	24	170.5	170.5	1	2.85	2.85	3585	3585	16-Aug	17-Aug	1			0.017	21
2	31.0	331.6	331.6	1	16.0	16.0	16014	16014	13-Jul	14-Jul	1			0.048	48
2	27.0	241.1	213.5	2	2.6	5.1	3599	7199	13-Jul	1-Sep	50			0.021	30
2	33.5	397.7	416.6	2	11.9	23.8	11111	22222	22-May	9-Jun	18			0.060	56
2	23.5	142.3	170.5	4	9.9	39.8	17551	70203	16-Jun	16-Aug	61			0.280	493
2	25.0	176.5	165.5	5	24.6	122.8	31791	158954	2-Jun	20-Jul	48			0.696	901
2	31.0	349.0	331.2	5	25.3	126.7	36539	182696	2-Jun	16-Aug	75			0.363	523
2	23.0	141.3	122.0	8	10.8	86.4	18668	149341	13-Apr	20-Jul	98			0.612	1057
2	26.5	200.9	249.5	9	13.3	119.6	19664	176977	13-Apr	24-Aug	133			0.595	881
2	29.5	341.4	267.4	9	22.3	200.7	43951	395556	28-Feb	24-Aug	177			0.588	1159

APPENDIX 6

The time untill hatch and first-feeding of flounder eggs and larvae at different constant temperatures.

Spawn date	Batch Temperature untill hatch	untill first feeding	Hatch date	First-feeding date	Days untill hatch	first-feeding	nr
23-May	2.2 ± 0.18	2.3 ± 0.18	8-Jun	26-Jun	16	34	
23-May	2.2 ± 0.18	2.3 ± 0.18	8-Jun	26-Jun	16	34	
11-May	2.3 ± 0.19	2.3 ± 0.2	30-May	11-Jun	19	31	
13-Apr	2.3 ± 0.13	2.3 ± 0.22	2-May	17-May	19	34	1
13-Apr	2.3 ± 0.13	2.3 ± 0.22	2-May	17-May	19	34	2
4-May	2.3 ± 0.21		22-May		18		
19-Apr	2.4 ± 0.26	2.4 ± 0.23	8-May	24-May	19	35	
10-Apr	2.4 ± 0.15		1-May		21		3
10-Apr	2.4 ± 0.15		1-May		21		4
16-Mar	6.1 ± 0.34		26-Mar		10		5
16-Mar	6.1 ± 0.34		26-Mar	3-Apr	10	18	1
16-Mar	6.1 ± 0.34	6.1 ± 0.26	25-Mar	3-Apr	9	18	3
16-Mar	6.1 ± 0.34	6.1 ± 0.26	26-Mar	3-Apr	10	18	4
13-Mar	6.3 ± 0.14	6.1 ± 0.27	23-Mar	29-Mar	10	16	9
13-Mar	6.3 ± 0.14		23-Mar	29-Mar	10	16	10
13-Mar	6.3 ± 0.14	6.1 ± 0.27	23-Mar	31-Mar	10	18	2
13-Mar	6.3 ± 0.14	6.1 ± 0.27	23-Mar	31-Mar	10	18	6
20-Mar	10.1 ± 0.07	10 ± 0.07	26-Mar	29-Mar	6	9	
20-Mar	10.1 ± 0.07	10 ± 0.07	25-Mar	30-Mar	5	10	1
27-Mar	10.2 ± 0.11	10 ± 0.15	3-Apr	7-Apr	7	11	
3-Apr	10.2 ± 0.18		9-Apr		6		
16-Mar	10.3 ± 0.17		22-Mar	27-Mar	6	11	6
13-Mar	10.3 ± 0.17	10 ± 0.16	21-Mar	23-Mar	8	10	7
16-Mar	10.3 ± 0.17	10 ± 0.17	23-Mar		7		
13-Mar	10.4 ± 0.15	10 ± 0.16	20-Mar	23-Mar	7	10	8
10-Mar	10.5 ± 0	10 ± 0.17	16-Mar	20-Mar	6	10	1
10-Mar	10.5 ± 0	10 ± 0.17	16-Mar	20-Mar	6	10	2
10-Mar	10.5 ± 0	10 ± 0.16	16-Mar	20-Mar	6	10	3

APPENDIX 7

Length of flounder larvae *(Platichtys flesus)* at hatch and at the first feeding stage.

Temperature (°C)	Length at hatch (mm)	Length at first feeding (mm)
2.2	3.40	
2.2	3.31	
2.3	2.81	
2.3	3.04	
2.3	2.72	
2.3	3.11	
2.3		4.20
2.3		4.24
2.3		3.97
2.3		4.18
2.3		4.11
2.4	3.26	
2.4	3.09	
2.4	3.06	
2.4		3.99
6.1	3.90	
6.1	3.75	
6.1	3.50	
6.1	3.99	
6.1		4.14
6.1		3.79
6.1		4.33
6.1		4.30
6.1		4.24
6.3	3.18	
6.3	3.02	
6.3	3.14	
10.1		4.16
10.1		3.57
10.2	4.40	
10.2		4.34
10.2		3.79
10.2		4.09
10.2		3.47
10.3	3.14	
10.3	3.14	
10.5	3.50	
10.5	3.61	
10.5		

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