

# Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea

Anders Nissling, Lars Westin, and Olle Hjerne



Nissling, A., Westin, L., and Hjerne, O. 2002. Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea. – ICES Journal of Marine Science, 59: 93–108.

The reproductive success and thus abundance and distribution of dab, plaice, and flounder in the Baltic Sea, a large brackish water area, is restricted by salinity. By measuring spermatozoa mobility and fertilisation rates at different salinities and determining the salinity at which eggs are neutrally buoyant, the salinity requirements for successful egg development were assessed. The results were used for the evaluation of potential spawning areas and for stock discrimination by analyses of differences in the salinity requirements of fish from different areas (ICES Subdivisions (SD) 23–28). The results suggest that there are two stocks of dab and successful reproduction may occur in the Sound (SD 23) and, occasionally, in the Arkona and Bornholm basins (SD 24 and SD 25). Opportunities for successful reproduction of plaice exist regularly in the Arkona and Bornholm basins and occasionally in the Gdansk and Gotland basins (SD 26 and SD 28). No differences in salinity requirements for fish from SD 24–28 suggest one stock of plaice in the Baltic proper. There are two different types of flounder, one with demersal eggs and the other with pelagic eggs. The former, constituting one distinct stock, may reproduce successfully as far north as the Bothnian Sea and the Gulf of Finland (SD 30 and SD 32), up to the 6 psu isohaline. For flounder with pelagic eggs, opportunities for the eggs to obtain neutral buoyancy suggest that successful reproduction may occur regularly in the Sound, the Arkona, and Bornholm basins as well as in the Gdansk and Gotland basins, and that there are three stocks of flounder with pelagic eggs.

© 2002 International Council for the Exploration of the Sea

Keywords: Baltic Sea, dab, egg buoyancy, flounder, plaice, reproduction, salinity, spermatozoa mobility, stock discrimination.

Received 22 November 2000; accepted 30 August 2001; published electronically 16 November 2001.

Anders Nissling and Lars Westin: Ar Research Station, Gotland University College, Fleringe, S-620 35 Fårösund, Sweden. Olle Hjerne: Department of Systems Ecology, Stockholm University, S-106 91 Stockholm, Sweden. Correspondence to A. Nissling: e-mail: [anders.nissling@hgo.se](mailto:anders.nissling@hgo.se)

## Introduction

Occurrence of a fish species in an area is determined by the availability of suitable habitats in terms of e.g. food availability, species interactions including predation and competition, and abiotic factors such as temperature, salinity, and oxygen content. A prerequisite for a species to colonise and inhabit an area is the potential to reproduce successfully, either within the area itself or in close proximity whereby presence of the species may be maintained by migration and/or passive dispersal of

young stages. The Baltic Sea, a large brackish water system inhabited by both freshwater and marine fish species, has passed through different stages since the last glacial period (10 000 years before present), from fresh water and marine environment to brackish water conditions, respectively. The selection pressure on marine species adapting to the Baltic Sea has probably been strong, with salinity acting as a major evolutionary force. Marine fishes unable to adapt to low salinity did not colonise the Baltic while individuals of species whose higher phenotypic plasticity enabled them to cope with

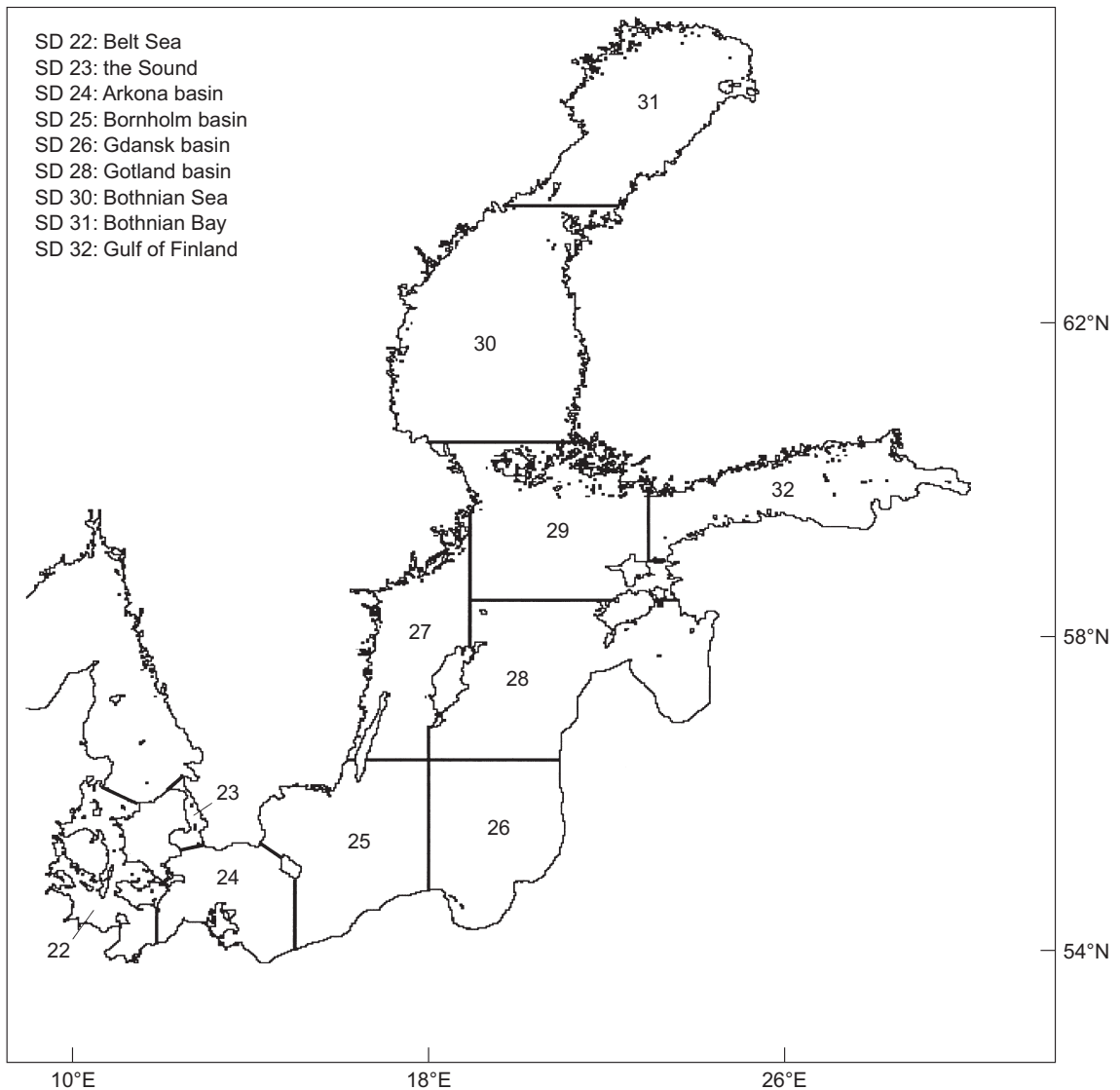


Figure 1. ICES Subdivisions (SD) in the Baltic Sea and names of areas referred to in the text.

low salinity succeeded, i.e. the occurrence and distribution of marine fishes in the Baltic Sea is to a large extent determined by salinity (see Voipio, 1981).

Salinity in the Baltic Sea [ICES Subdivisions (SD) 24–32] (Figure 1) is maintained by inflows of saline water from the North Sea through the Sound and the Belt Sea. Consequently, salinity in the Baltic Sea decreases from the west to the east and from the south to the north, ranging between 8 and 3 psu in the surface water. However, salinity varies not only horizontally but also vertically, due to a halocline at 50–70 m in the deep basins in the Baltic proper (SD 24–28). Salinity below the halocline ranges between ~10 and ~22 psu. Exchange between the surface and bottom water is restricted, and the renewal of the bottom water is mainly

driven by major saline water inflows into the Baltic Sea. Inflow events occur only under certain meteorological conditions, and are highly irregular. Stagnant water conditions in the bottom layers, accompanied by a decrease in salinity and oxygen concentration, may prevail for years (Fonselius, 1962; Franck *et al.*, 1987; Matthäus and Lass, 1995). Spawning of marine fishes with pelagic eggs in the Baltic Sea is, due to low saline surface water, restricted to the deep basins, i.e. the Arkona (SD 24), Bornholm (SD 25), Gdansk (SD 26) and Gotland (SD 28) basins (Figure 1). Conditions for successful reproduction in the deep basins are thus governed by the highly irregular inflow events, and those very variable spawning conditions have implications for recruitment and stock development.

Table 1. Number and length range (cm) of males and females of dab, plaice, and flounder sampled in each ICES Subdivision (SD).

	SD 23		SD 24		SD 25		SD 28	
	Males	Females	Males	Females	Males	Females	Males	Females
<b>Dab</b>								
Number	12	3	14	11	3	1	—	—
Length	24–29	29–35	22–32	23–29	21–24	27		
<b>Plaice</b>								
Number	—	—	10	3	14	11	10	3
Length			22–36	34–47	21–33	29–43	27–40	37–46
<b>Flounder</b>								
Number	9	4	11	20	15	10	7	10
Length	22–32	24–33	—	28–37	24–32	23–33	23–31	29–46

Table 2. Duration of spermatozoa mobility (minutes)  $\pm$  standard deviation of dab from ICES Subdivisions (SD) 23, 24 and 25 at different salinities (psu).

Salinity	SD 23	SD 24	SD 25
10	—	0 $\pm$ 0	0 $\pm$ 0
11	0 $\pm$ 0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1
12	0.2 $\pm$ 0.3	0.6 $\pm$ 0.5	1.2 $\pm$ 0.6
13	0.9 $\pm$ 0.7	1.5 $\pm$ 0.5	1.3 $\pm$ 0.4
14	1.6 $\pm$ 1.0	—	—
15	1.5 $\pm$ 0.4	1.8 $\pm$ 0.4	2.1 $\pm$ 0.6
20	2.0 $\pm$ 0.4	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2
30	2.2 $\pm$ 0.3	1.7 $\pm$ 0.3	2.0 $\pm$ 0.4

Adaptation to environmental conditions occurs as both phenotypic plasticity and by genetic selection. Genetic selection resulting in a higher proportion of offspring from individuals adapting to a situation more successfully than others may imply changes in genotypic frequencies over generations. Assuming that salinity is a strong evolutionary force acting on marine fish populations colonising the Baltic, differences in the salinity requirements for factors leading to successful reproduction such as egg buoyancy and the potential for fertilisation at low salinities, may be used not only to evaluate potential spawning areas but also for stock discrimination [see an earlier investigation on cod, *Gadus morhua*, showing that characteristics such as salinity of spermatozoa activation and the salinity at which eggs obtain neutral buoyancy are specific to populations (Nissling and Westin, 1997)].

The distribution of three Pleuronectids, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the Baltic Sea is summarised by Bagge (1981). Flounder is distributed in all parts of the Baltic except for the eastern part of Gulf of Finland (SD 32) and the Bothnian Bay (SD 31), whereas dab and

plaice are restricted to the southern parts. Dab occurs only in SD 24 and SD 25, whereas the regular area of distribution of plaice extends eastwards to the Gulf of Gdansk (SD 26) and northwards to the Gotland area (SD 28). The spawning of dab, plaice, and flounder in the south, where all three species produce pelagic eggs, is restricted to the deep basins, whereas flounder in the northern parts produce demersal eggs and spawn in shallower areas (e.g. Molander, 1925). Being widely distributed, flounder is the most important species in the Baltic flatfish fishery (Bagge, 1981) although fluctuations in stock abundance occur, perhaps due to environmental variability (Solemdal, 1970; Ojaveer *et al.*, 1985). In the southern Baltic fishery, flatfishes were main targets early in the 20th century. However, their yield decreased significantly during the 1930s, probably due to both changed hydrographic conditions and overfishing (Temming, 1989a).

In the present study the effect of salinity on the reproductive success of the three Pleuronectids in the Baltic Sea was studied. Spermatozoa mobility, fertilisation, and egg development at different salinities were assessed, and the salinity at which eggs are neutrally buoyant (hereafter referred to as SNB) was determined. The ability to remain buoyant and thus to avoid the low oxygen levels in the bottom layers, is crucial for the development of pelagic eggs in the Baltic (Strodtmann, 1918; Kändler, 1944; Hohendorf, 1968; Nissling *et al.*, 1994).

## Material and methods

The study includes measurements on fish collected from ICES Subdivisions 23, 24, 25, and 28 (Figure 1) during the period 1991–1998. Fish were caught during trawl surveys by RV “Argos” in SDs 23, 24, and 25 in February–April, and by local fishermen in SD 28 in March–May. In total 44 dab, 51 plaice, and 86 flounder

Table 3. Lowest salinity (psu)  $\pm$  standard deviation of spermatozoa activation of dab, plaice, and flounder in each ICES Subdivision (SD).

	SD 23	SD 24	SD 25	SD 28
<b>Dab</b>				
Average	12.9 $\pm$ 0.8	11.9 $\pm$ 0.7	11.7 $\pm$ 0.6	—
Median	13	12	12	—
Minimum	12	11	11	—
<b>Plaice</b>				
Average	—	11.5 $\pm$ 0.8	11.8 $\pm$ 1.0	10.8 $\pm$ 0.7
Median	—	12	12	10.5
Minimum	—	10	10	9
<b>Flounder</b>				
Average	11.6 $\pm$ 1.0	11.8 $\pm$ 0.6	10.3 $\pm$ 1.3	3.4 $\pm$ 0.3
Median	11	12	10	3
Minimum	10	10	9	3

Table 4. Salinity range (psu) at which egg batches (number) of dab from ICES Subdivisions (SD) 23, 24, and 25 were neutrally buoyant, determined by egg specific gravity measurements.

Salinity	SD 23	SD 24	SD 25
19–19.5		1	
19.5–20		1	
20–20.5		2	
20.5–21		1	
21–21.5		2	
21.5–22			
22–22.5		1	
22.5–23		1	1
23–23.5			
23.5–24			
24–24.5			
25–25.5			
25.5–26	2		
26–26.5	1		
26.5–27		1	
27–27.5		1	

were used (Table 1). Measurements were performed either directly on spawning fish caught during the surveys (in SDs 23, 24, and 25) or after transportation to the Ar laboratory, Gotland, Sweden, where the fish were kept in tanks ( $\sim 12 \text{ m}^3$ ) provided with running water of  $\sim 7$  psu until the start of spawning (fish from SD 28). To evaluate the phenotypic potential for adaptation to lower salinities, measurements were also performed on plaice from SD 24–25 after transportation to the Ar laboratory (SD 28) and maintenance at 7 psu for 1 month prior to spawning. Plaice from SD 24–25 did not produce eggs of lower specific gravity than those caught during surveys, i.e. the eggs were neutrally buoyant at the same salinity, or display significant lower salinity of spermatozoa activation when kept at 7 psu (see below).

Accordingly, these fish were treated as one sample, irrespective of sampling method.

#### Sperm mobility and egg development

Sperm mobility was measured at approximately  $7^\circ\text{C}$  under a microscope at  $250\times$  magnification. Spermatozoa activity was recorded as swimming, vibrating or immobile. If swimming spermatozoa were observed, the time until locomotory activity ceased (i.e. until the spermatozoa became vibrating or immobile) was recorded. For each determination, semen was sampled directly from the male using a dry Pasteur pipette. For each salinity tested, one droplet of semen was diluted in  $\sim 5$  ml water prepared from synthetic seasalt (hw Marine mix, Wiegandt, Germany) and deionised water, and observation began immediately. For each male, measurements started with a high salinity followed by subsequently lower salinities until spermatozoa mobility ceased.

Fertilisation success was assessed by mixing eggs, obtained by stripping and semen collected with a dry Pasteur pipette. Water of different salinities was prepared from filtered ( $0.2 \mu\text{m}$  cartridge filter) Baltic Sea water (7 psu) with addition of synthetic seasalt or dilution with deionised water. Eggs from one female and semen from one male were used in each trial. One droplet of semen was mixed with  $\sim 100$  ml water and a spoonful of eggs ( $\sim 1000$  eggs) added immediately. After  $\sim 2$  h the eggs were rinsed in water of the respective salinity and incubated further at  $7^\circ\text{C}$ . The number of fertilised eggs was counted 6–8 h after fertilisation. Eggs displaying cell cleavage were considered fertilised as opposed to activated eggs, i.e. eggs with one germinal cell (Howell *et al.*, 1991), and eggs without visible cells. Only clear/transparent eggs were considered. To obtain information about hatching success at extreme salinities, eggs of flounder caught in SD 28 were incubated in

Table 5. Salinity of neutral egg buoyancy (SNB) (psu) and egg size (mm)  $\pm$  standard deviation of dab, plaice, and flounder in each ICES subdivision (SD).

	SD 23	SD 24	SD 25	SD 28
<b>Dab</b>				
SNB average	26.4 $\pm$ 0.6 <sup>a</sup>	20.8 $\pm$ 1.1 <sup>a</sup>	22.6	—
SNB range	25.8–27.1 <sup>a</sup>	19.2–22.6 <sup>a</sup>	—	—
<b>Plaice</b>				
SNB average	—	15.3 $\pm$ 0.4	15.7 $\pm$ 0.9	16.7 $\pm$ 1.3
SNB range	—	15.0–15.7	14.0–17.7	16.3–18.2
Egg size	—	1.90 $\pm$ 0.06	1.87 $\pm$ 0.18	1.88 $\pm$ 0.16
<b>Flounder</b>				
SNB average	26.1 $\pm$ 0.8	15.2 $\pm$ 1.9	13.9 $\pm$ 1.5	20.3 $\pm$ 1.1
SNB range	25.0–26.7	13.1–17.7	11.8–16.7	18.0–21.5
Egg size	1.12 $\pm$ 0.07	1.34 $\pm$ 0.04	1.43 $\pm$ 0.06	0.99 $\pm$ 0.05

<sup>a</sup>Includes (SD 23) or excludes (SD 24) egg buoyancy measurements from two fish caught in the western part of SD 24 (see Material and methods).

Table 6. Estimated SNB, i.e. egg specific gravity expressed in psu, in each ICES Subdivision (SD), at which 5, 2.5, and 1% of spawned eggs of dab, plaice, and flounder with pelagic eggs would be neutrally buoyant (based on average egg specific gravity in present investigation and assumed normal distribution), and minimum salinity for successful fertilisation for flounder with demersal eggs (SD 28) respectively.

	5%	2.5%	1%	Lowest observed value
p	0.90	0.95	0.98	
Z	1.645	1.96	2.33	
Dab SD 24–(25)	18.7	18.3	17.8	19.2
Plaice SD 24–28	13.6	13.1	12.6	14.0
Flounder SD 24	13.0	12.5	12.0	13.1
Flounder SD 25	11.7	11.2	10.7	11.8
Flounder SD 28				$\geq 6$

300 ml water of 6 psu at 7°C until hatching, with daily exchange of water.

### Egg buoyancy and egg size

Eggs were obtained by stripping and the egg specific gravity of unfertilised eggs was determined at 7°C, using a density gradient column according to Coombs (1981), and the salinity at which eggs were neutrally buoyant (SNB) calculated. As evident from investigations on cod (Nissling and Westin, 1991a), there is no difference between the buoyancy of unfertilised and one-day-old fertilised eggs. The positions of the eggs were compared with the positions of six density floats of known specific gravity (correlation coefficient of the density floats  $>0.99$  at all measurements). For each measurement 10–15 eggs were used. Egg size was measured under a stereomicroscope at 50 $\times$  magnification using a micrometer

scale. Approximately 15 eggs were measured in each determination.

### Calculation of variation in egg buoyancy

To evaluate opportunities for eggs of respective species/population to obtain neutral buoyancy, i.e. to float, in different areas, the salinity at which 5, 2.5, and 1% of the eggs/egg batches in the population would be neutrally buoyant was estimated. By using normal distribution and standard deviation (s.d.) ( $p=0.90, 0.95, 0.98$ ) the lowest egg specific gravity, obtained at the left hand tail of the distribution, was used to calculate the SNB at the respective level. As the number of egg batches were in some cases few, the variation in egg buoyancy for Baltic cod (Vallin and Nissling, 2000) was used to estimate the variation in SNB for the flatfish populations. As most continuous parameters are known to be approximately

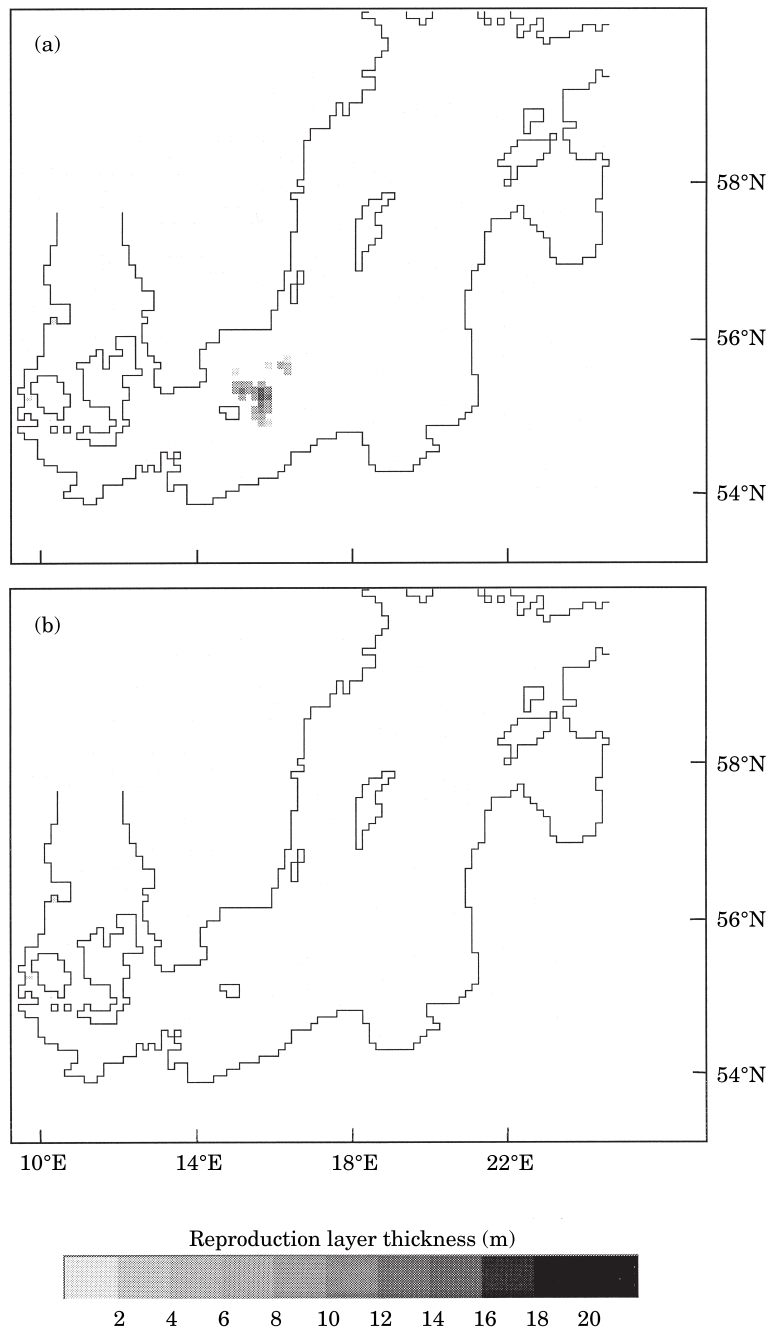


Figure 2. Distribution of reproductive volume ( $\geq 17.8$  psu;  $\geq 2$  ml  $O_2/l$ ) of dab in the Baltic during (a) favourable (March–July 1952) and (b) unfavourable (March–July 1988) environmental conditions respectively based on salinity requirements in the present investigation. N.B. Calculation of reproductive volume in 1952 was not possible in SD 24 due to lack of data.

normally distributed, a goodness of fit was applied to data for Baltic cod (neutral egg buoyancy at  $14.5 \pm 1.4$  psu). Egg buoyancy was found to be normally distributed ( $\chi^2=10.06$ , d.f.=5,  $0.05 < p < 0.1$ ) for this species, with 90% corresponding to  $\pm 1.645$  s.d., 95% to  $\pm 1.96$  s.d., and 98% to  $\pm 2.33$  s.d. (z-distribution).

Bartlett's test for homogeneity of variances (Dixon and Massey, 1983) showed equal variance in neutral egg buoyancy for cod and the respective flatfish populations in present study ( $F_{3,\infty}=0.861$ ;  $0.5 < p < 1.0$ ). Hence, assuming an approximately normal distribution also for the egg buoyancy of flatfishes, the above z-distribution

Table 7. Duration of spermatozoa mobility (minutes)  $\pm$  standard deviation of plaice from ICES Subdivisions (SD) 24, 25, and 28 at different salinities (psu).

Salinity	SD 24	SD 25	SD 28
8	—	—	0 $\pm$ 0
9	0 $\pm$ 0	0 $\pm$ 0	0.5 $\pm$ 0.9
10	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	1.1 $\pm$ 1.6
11	0.4 $\pm$ 0.6	0.2 $\pm$ 0.4	1.0 $\pm$ 0.8
12	1.5 $\pm$ 1.4	0.8 $\pm$ 0.7	2.5 $\pm$ 1.6
13	2.0 $\pm$ 0.8	1.2 $\pm$ 1.1	—
14	2.1 $\pm$ 0.5	1.5 $\pm$ 0.8	—
15	4.9 $\pm$ 0.2	3.8 $\pm$ 1.2	4.1 $\pm$ 0.9
20	3.1 $\pm$ 0.8	4.4 $\pm$ 0.7	3.5 $\pm$ 1.2
30	2.5 $\pm$ 0.1	3.6 $\pm$ 1.4	—

Table 8. Salinity range (psu) at which egg batches (number) of plaice from ICES Subdivisions (SD) 24, 25, and 28 were neutrally buoyant, determined by egg specific gravity measurements.

Salinity	SD 24	SD 25	SD 28
14–14.5		1	
14.5–15			
15–15.5	2	3	
15.5–16	1	4	1
16–16.5		1	1
16.5–17		1	
17–17.5			
17.5–18		1	
18–18.5			1

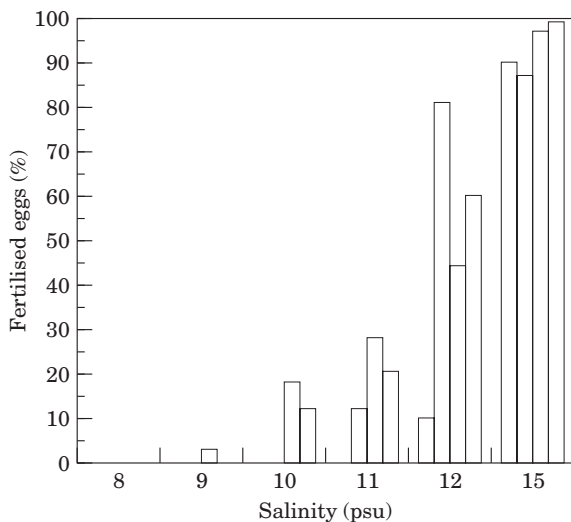


Figure 3. Percentage of fertilised eggs of plaice after fertilisation at different salinities (four set ups).

was used to estimate the variation in neutral egg buoyancy for the respective species/population.

### Differences between areas

Differences in salinity requirements of spermatozoa activation between fish of each species caught in different areas were tested using bootstrapping. Salinity of spermatozoa activation obtained for each male was randomised among the groups (areas) 100 times using EXCEL. Differences (absolute values) among the created groups were then compared with the actual difference. If the actual difference was higher than the 95th percentile of the created differences obtained from the randomisations,  $H_0$  (no difference between groups/areas) was rejected. Differences in average neutral egg

buoyancy between areas for fish of respective species were tested using Student's t-test (STATVIEW).

### Calculations of habitats suitable for spawning

From data in the Baltic Environmental Database (BED) and using the Data Assimilation System software (DAS; Sokolov *et al.*, 1997) we calculated the reproduction volume (dab, plaice, and flounder with pelagic eggs) and reproduction area (flounder with demersal eggs). We used the salinity reproductive limits presented here and assumed the minimum required oxygen concentration to be 2 ml  $O_2/l$  (cf. Wieland *et al.*, 1994). Inspection of preliminary maps revealed stations with big gaps between sampling depths, resulting in unrealistic results as a consequence of the interpolation procedure (cf. Sokolov *et al.*, 1997). After removal of these stations, we constructed maps with examples of the spatial distribution of habitat suitable for reproduction during favourable and unfavourable conditions.

## Results

### Dab

Duration of spermatozoa mobility at different salinities is shown in Table 2. Activation of spermatozoa occurred at 11–14 psu with an increase in duration of mobility with salinity. Spermatozoa activation occurred at an average salinity of  $12.9 \pm 0.8$  psu for fish from SD 23, at  $11.9 \pm 0.7$  psu in SD 24, and at  $11.7 \pm 0.6$  psu in SD 25 (Table 3) reflecting a slight adaptation to lower salinity conditions. Bootstrapping analysis revealed a significant difference ( $p < 0.01$ ) in salinity of spermatozoa activation between fish from SD 23 and SD 24.

SNB for fish caught in different areas is shown in Table 4. It is evident that there are two distinct groups. One group consists of eggs from females caught in SD 23 and the westernmost part of SD 24 (trawl stations at 54.55°N, 13.17°E and 55.11°N 13.15°E), and the other of



eggs from females in central SD 24 (east of 55.12°N 13.88°E) and SD 25 ( $t=2.57$ ,  $d.f.=12$ ,  $p<0.05$ ), with SNB averaging  $26.4 \pm 0.6$  and  $21.0 \pm 1.2$ , respectively (Table 5).

Opportunities for successful spawning are limited by the possibilities of obtaining neutral egg buoyancy. As given in Table 6 the salinity at which 1% of the egg batches in the population will obtain neutral egg buoyancy (i.e. eggs with the lowest egg specific gravity) is 17.8 psu. This salinity occurs only occasionally in the Arkona and the Bornholm basins, e.g. in the Bornholm basin in the early to mid-1950s, the mid-late 1960s and after the inflow events in the late 1970s and 1993–1994 (Fonselius, 1962; Matthäus, 1993; Matthäus *et al.*, 1994). Figure 2 shows an example of the distribution of habitat suitable for dab reproduction during favourable and unfavourable conditions, respectively. The results suggest that successful reproduction of dab may take place not only in the Sound (SD 23), but occasionally also in the Arkona basin (SD 24) and the Bornholm basin (SD 25), whereas salinity is too low in the Gdansk and Gotland basins (SD 26 and SD 28). Comparison of SNB and salinity of spermatozoa activation for fish from areas SD 23–25 suggest two populations/stocks in the area, one in the Sound and one in the Baltic proper.

### Plaice

Plaice from SD 24–25, transported to the laboratory and kept at 7 psu, did not produce eggs of lower specific gravity, SNB at  $15.8 \pm 0.8$  psu ( $n=9$ ), than those caught during surveys, SNB at  $15.0 \pm 0.7$  psu ( $n=4$ ), or display significant lower salinity of spermatozoa activation [10–12 psu ( $n=7$ ) and 10–14 ( $n=20$ ), respectively]. Accordingly, the fish were treated as one sample irrespective of sampling method.

Table 7 shows duration of spermatozoa mobility at different salinities. Swimming spermatozoa were recorded at 9–12 psu, irrespective of area, and the duration of mobility increased with salinity up to 15 psu. The salinity of spermatozoa activation for males from SD 28 ( $10.8 \pm 0.7$  psu), was lower than for males from SD 24 and SD 25,  $11.5 \pm 0.8$  and  $11.8 \pm 1.0$  psu respectively ( $p<0.05$ ; bootstrapping).

Fertilisation experiment performed at salinities between 8 and 15 psu, with four trials with different males and females from SD 24–28, showed a low percentage of fertilised eggs at low salinities. In accordance with spermatozoa activity, successful fertilisation was low and variable at 9–12 psu and increased with salinity up to 15 psu. However, probably due to differences in salinity of spermatozoa activation, the lowest salinity required for fertilisation varied (Figure 3).

SNB was on average  $15.3 \pm 0.4$ ,  $15.7 \pm 0.9$ , and  $16.7 \pm 1.3$  psu for fish from SD 24, 25, and 28, respectively (Table 5) with no significant differences between

areas ( $t=0.668/1.834/1.683$ ,  $d.f.=13/4/13$ ,  $p>0.05$ ) (Table 8). Also, no differences in egg sizes were recorded ( $1.90 \pm 0.06$ ,  $1.87 \pm 0.18$  and  $1.88 \pm 0.16$  mm, respectively).

Lower limits for successful spawning at 12.6–13.6 psu (Table 6) imply that eggs may develop successfully from time to time in the eastern deep basins. In SD 26 a salinity of  $\sim 13.5$  psu occurred in the bottom layers during the early to mid-1950s, more or less regularly during the 1960s as well as in the mid-late 1970s and after the inflow in 1993–1994. In the deepest parts of SD 28 a salinity of  $\sim 13$  psu occurred during the 1950s and a salinity of  $\sim 12.5$  psu during the 1960s and in the late 1970s (Fonselius, 1962; Matthäus *et al.*, 1994). Accordingly, with respect to salinity requirements, opportunities for successful spawning of plaice exist regularly in the Arkona basin (SD 24) and the Bornholm basin (SD 25) but occasionally also in the Gdansk and Gotland basins (SD 26 and SD 28) as shown in Figure 4, which illustrates the distribution of reproductive volumes at favourable and unfavourable conditions, respectively. Evaluation of salinity of spermatozoa activation and SNB revealed no difference between areas (SD 24–28) (N.B. there were no samples from SD 23), suggesting only one population/stock in the Baltic proper.

### Flounder

Spermatozoa mobility at different salinities is shown in Tables 9 and 10. For flounders with pelagic eggs (Table 9) activation of spermatozoa occurred at salinities ranging from 9–12 psu, with an increase in the duration of mobility with salinity. Males from SD 25 displayed, on average, a significantly lower salinity of spermatozoa activation [ $10.3 \pm 1.3$  psu compared to  $11.6 \pm 1.0$  and  $11.8 \pm 0.6$  psu for males from SD 23 and SD 24, respectively ( $p<0.01$ , bootstrapping)] (Table 3). For flounders with demersal eggs (Table 10) mobile spermatozoa were recorded at 3–4 psu with significantly higher duration of spermatozoa mobility at  $\geq 6$  psu. However, a fertilisation experiment with six trials using different males and females from SD 28, showed no fertilisation at salinities  $<6$  psu and an increase in fertilisation rate with salinity up to 10–15 psu. Eggs fertilised and incubated at 6 psu hatched successfully, with normally developed larvae. At 6–7 psu fertilisation rates were both low and variable (Figure 5) suggesting highly variable fertilisation success in areas inhabited by flounders with demersal eggs.

Measurements of SNB (Table 11) revealed significant differences between all areas (SD 23–28) (Table 12) with SNB at on average  $26.1 \pm 0.8$ ,  $15.2 \pm 1.9$ ,  $13.9 \pm 1.5$ , and  $20.3 \pm 1.1$  psu for SD 23, 24, 25, and 28, respectively (Table 5). Along with the decrease in SNB (lower egg specific gravity), egg size increased significantly ( $1.12 \pm 0.07$ ,  $1.34 \pm 0.04$ , and  $1.43 \pm 0.06$  mm for eggs from females caught in SD 23, 24, and 25, respectively).



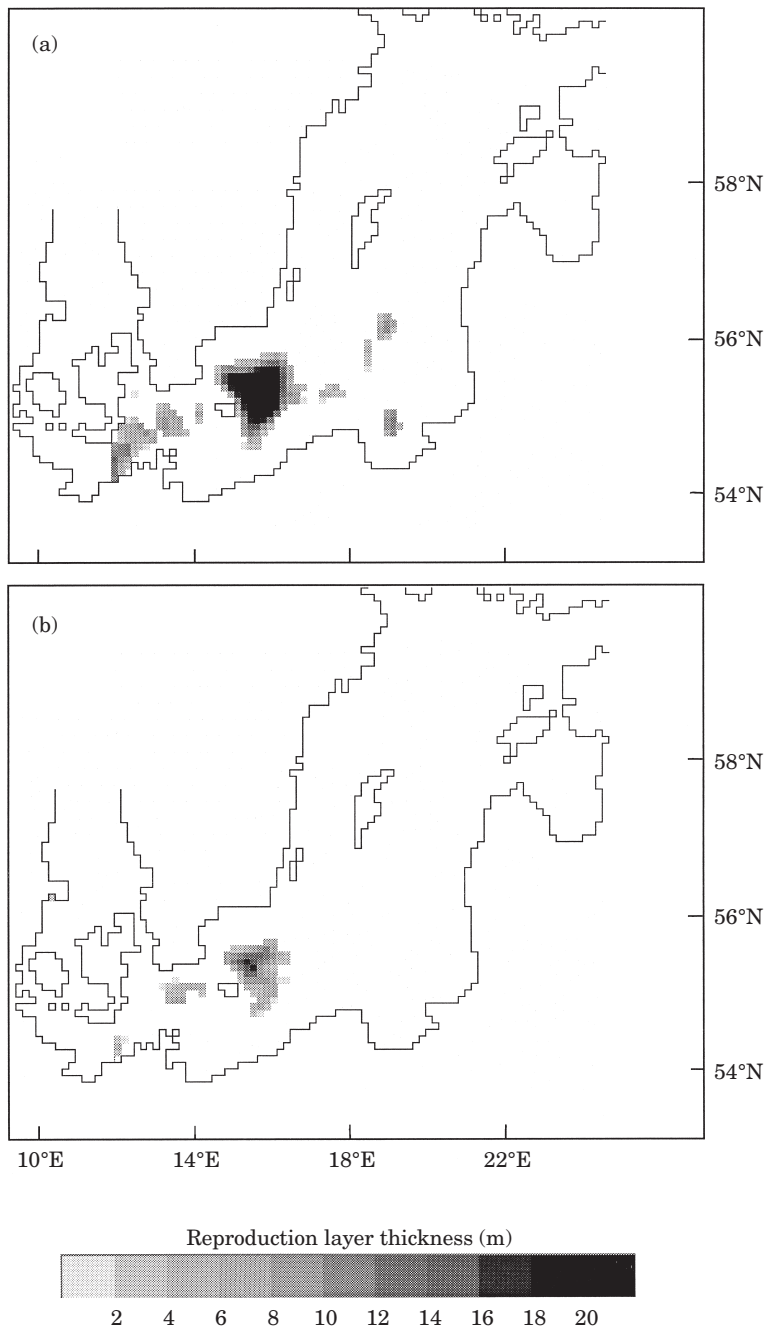


Figure 4. Distribution of reproductive volume ( $\geq 12.6$  psu;  $\geq 2$  ml  $O_2/l$ ) of plaice in the Baltic during (a) favourable (December 1976–April 1977) and (b) unfavourable (December 1987–April 1988) environmental conditions, respectively, based on salinity requirements in the present investigation.

However, flounder with demersal eggs caught in SD 28, displayed intermediate egg specific gravity but the smallest eggs;  $0.99 \pm 0.05$  mm.

SNB measurements together with spawning habits suggest that flounder in the Baltic Sea can be separated into three populations/stocks. Flounder with demersal

eggs constitute one distinct population, whereas those with pelagic eggs may be separated into two populations, one in SD 23 and the other in SD 24–25. For both salinity of spermatozoa activation as well as SNB and egg size, significant differences were recorded between fish from SD 24 and SD 25, indicating two

Table 9. Duration of spermatozoa mobility (minutes)  $\pm$  standard deviation of flounder from ICES Subdivisions (SD) 23, 24, and 25 at different salinities (psu).

Salinity	SD 23	SD 24	SD 25
8	—	—	0 $\pm$ 0
9	0 $\pm$ 0	0 $\pm$ 0	0.3 $\pm$ 0.3
10	0.1 $\pm$ 0.3	0.1 $\pm$ 0.1	0.4 $\pm$ 0.3
11	0.8 $\pm$ 0.8	0.1 $\pm$ 0.2	0.6 $\pm$ 0.7
12	1.2 $\pm$ 0.9	1.2 $\pm$ 0.6	2.0 $\pm$ 0.5
13	2.5 $\pm$ 1.3	—	—
14	3.2 $\pm$ 1.1	—	—
15	3.1 $\pm$ 0.8	2.4 $\pm$ 0.7	2.1 $\pm$ 0.7
30	—	2.1 $\pm$ 0.7	2.4 $\pm$ 0.9

Table 10. Duration of spermatozoa mobility (minutes)  $\pm$  standard deviation of flounder from ICES Subdivision (SD) 28 at different salinities (psu).

Salinity	SD 28
3	0.2 $\pm$ 0.1
4	0.3 $\pm$ 0.1
5	0.3 $\pm$ 0.1
5.5	0.4 $\pm$ 0.3
6	1.2 $\pm$ 0.5
7	2.0 $\pm$ 0.7
10	2.2 $\pm$ 0.4
15	2.9 $\pm$ 0.3

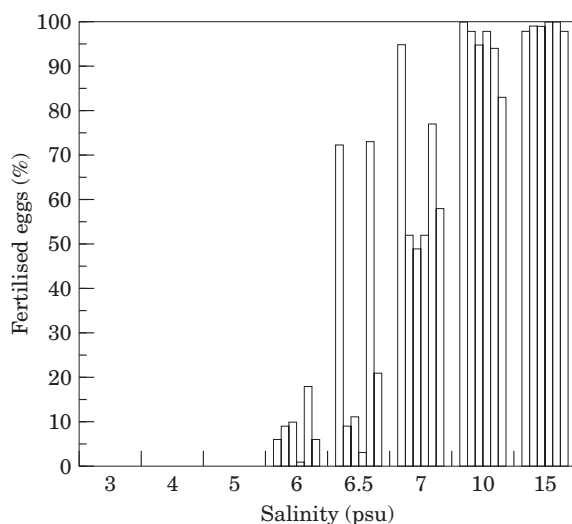


Figure 5. Percentage of fertilised eggs of flounder from SD 28 after fertilisation at different salinities (six set ups).

populations. However, differences are small and a direct adaptation to lower salinity conditions can not be excluded.

Considering salinity requirements and the potential for successful reproduction, flounder producing

demersal eggs may reproduce successfully north- and eastwards according to the 6 psu isohaline. For flounder producing pelagic eggs, reproduction is limited by opportunities for the eggs to float. According to the lower salinity limits for SNB given in Table 6, flotation can be achieved in the eastern deep basins, where the minimum salinity requirements of 10.7–11.7 psu are fulfilled (e.g. Fonselius, 1962; Mattäus *et al.*, 1994).

With respect to salinity requirements for reproduction, flounder display the highest degree of adaptation to the Baltic Sea brackish water of the Pleuronectid species. As two different types of flounder exist in the Baltic Sea, i.e. those with demersal eggs and those with pelagic eggs, successful reproduction may occur as far north as in the Bothnian Sea and Gulf of Finland (SD 30 and SD 32) (Figure 6). Considering flounder with pelagic eggs, SNB suggest that successful egg development occurs regularly in the Sound, the Arkona basin and the Bornholm basin (SD 23–25) but may also occur in the easternmost deep areas, the Gdansk and Gotland basins (SD 26 and SD 28) as shown in Figure 7.

## Discussion

For successful spawning in brackish water conditions both the spermatozoa and the eggs have to cope with low salinities. As spermatozoa in marine teleosts are known to be activated at hyperosmotic conditions after discharge into the water (Morisawa and Suzuki, 1980; Stoss, 1983) fertilisation can be affected in the brackish water conditions occurring in the Baltic Sea (see Solemdal, 1970; Nissling and Westin, 1997). Although successful egg development of marine teleosts may occur at low salinities (Marx and Henschel, 1941; Solemdal, 1967; Westernhagen, 1970; Nissling and Westin, 1991b) this may include premature hatching and thus decreased viability of the larvae [Holliday, 1965 (plaice, cod); Nissling and Westin, 1991b (cod)]. A major problem for marine teleosts with pelagic eggs in the Baltic Sea is to produce eggs that can float in the low salinity (density) brackish water. Adaptation to obtain neutral egg buoyancy in less saline water includes an increase in size and water content (Strodtmann, 1918; Mielck and Künne, 1935; Kändler, 1944; Solemdal, 1967; Kjesbu *et al.*, 1992; Thorsen *et al.*, 1996), i.e. flatfishes inhabiting the Baltic Sea display a decrease in SNB (egg specific gravity) compared to their marine counterparts (Lönning and Solemdal, 1972; Solemdal, 1973).

The present study suggests that adaptation for successful spawning of dab, plaice, and flounder in the Baltic Sea is not restricted by spermatozoa activation but by the ability of the egg to develop at low salinities. For flounder with demersal eggs spermatozoa activation occur at 3–4 psu but for fertilisation and egg

Table 11. Salinity range (psu) at which egg batches (number) of flounder from ICES Subdivisions (SD) 23, 24, 25, and 28 were neutrally buoyant, determined by egg specific gravity measurements.

Salinity	SD 23	SD 24	SD 25	SD 28
11.5–12			1	
12–12.5				
12.5–13			2	
13–13.5		2	1	
13.5–14			1	
14–14.5		6	2	
14.5–15		2	1	
15–15.5		1	1	
15.5–16		3		
16–16.5		3		
16.5–17		2	1	
17–17.5		1		
17.5–18		1		
18–18.5				1
18.5–19				1
19–19.5				
19.5–20				1
20–20.5				
20.5–21				5
21–21.5				1
21.5–22				1
25–25.5	1			
25.5–26	1			
26–26.5				
26.5–27	2			

Table 12. Differences in SNB (egg specific gravity) of flounder from different ICES Subdivisions (SD) (Student's t-test).

SD	d.f.	t	p
23 and 24	22	15.04	<0.001
23 and 25	12	15.43	<0.001
23 and 28	12	9.27	<0.001
24 and 25	28	2.46	<0.05
24 and 28	28	9.89	<0.001
25 and 28	18	10.79	<0.001

development a salinity  $\geq 6$  psu is required. For dab, plaice, and flounder with pelagic eggs, activation of spermatozoa and fertilisation occurred at an average of 10–13 psu, whereas an average salinity 13.9–26.1 psu was required to obtain the neutral egg buoyancy crucial for egg survival up to hatching.

Looking at salinity requirements for the spermatozoa activation and neutral egg buoyancy respectively, the largest adaptation of Baltic Pleuronectids compared to their marine counterparts occur for the egg. The salinity required for spermatozoa activation (average 10–13 psu) for dab, plaice, and flounder with pelagic eggs is the same as the minimum salinity necessary for successful fertilisation of flounder off Norway, 11 psu (Solemdal, 1970). Regarding egg buoyancy (egg specific gravity) on

the other hand, Baltic populations of dab, plaice, and flounder have significantly larger and more buoyant eggs (e.g. Strodtmann, 1918; Hohendorf, 1968; Lönning and Solemdal, 1972; Solemdal, 1973; here). Flounder producing demersal eggs is an exception. For this population, activation of spermatozoa is a major breakthrough as spermatozoa are activated at salinities as low as 3–4 psu. Freedom from the need to produce buoyant eggs has opened up a new niche, making flounder the most abundant Pleuronectid in the Baltic.

Adaptation to brackish water conditions may occur as both phenotypic plasticity and by genetic selection. For teleosts it has been shown that osmolality in seminal plasma and ovarian fluid is lower in brackish water conditions than in marine environments; e.g. Strodtmann (1918); Nissling and Westin (1997) (cod); Hohendorf (1968) (cod and plaice). A phenotypic adaptation with lower internal osmolality may affect egg buoyancy and salinity of spermatozoa activation. However, despite significantly changed osmolality in ovarian fluid and seminal plasma, transference experiments have showed only minor changes in egg buoyancy [Solemdal (1971; 1973) (flounder); Nissling and Westin (1997) (cod)] after transference from brackish to marine conditions or *vice versa*, as well as in salinity of spermatozoa activation [Nissling and Westin (1997) (cod)]. This suggests that characters like salinity of spermatozoa activation and egg specific gravity, i.e.

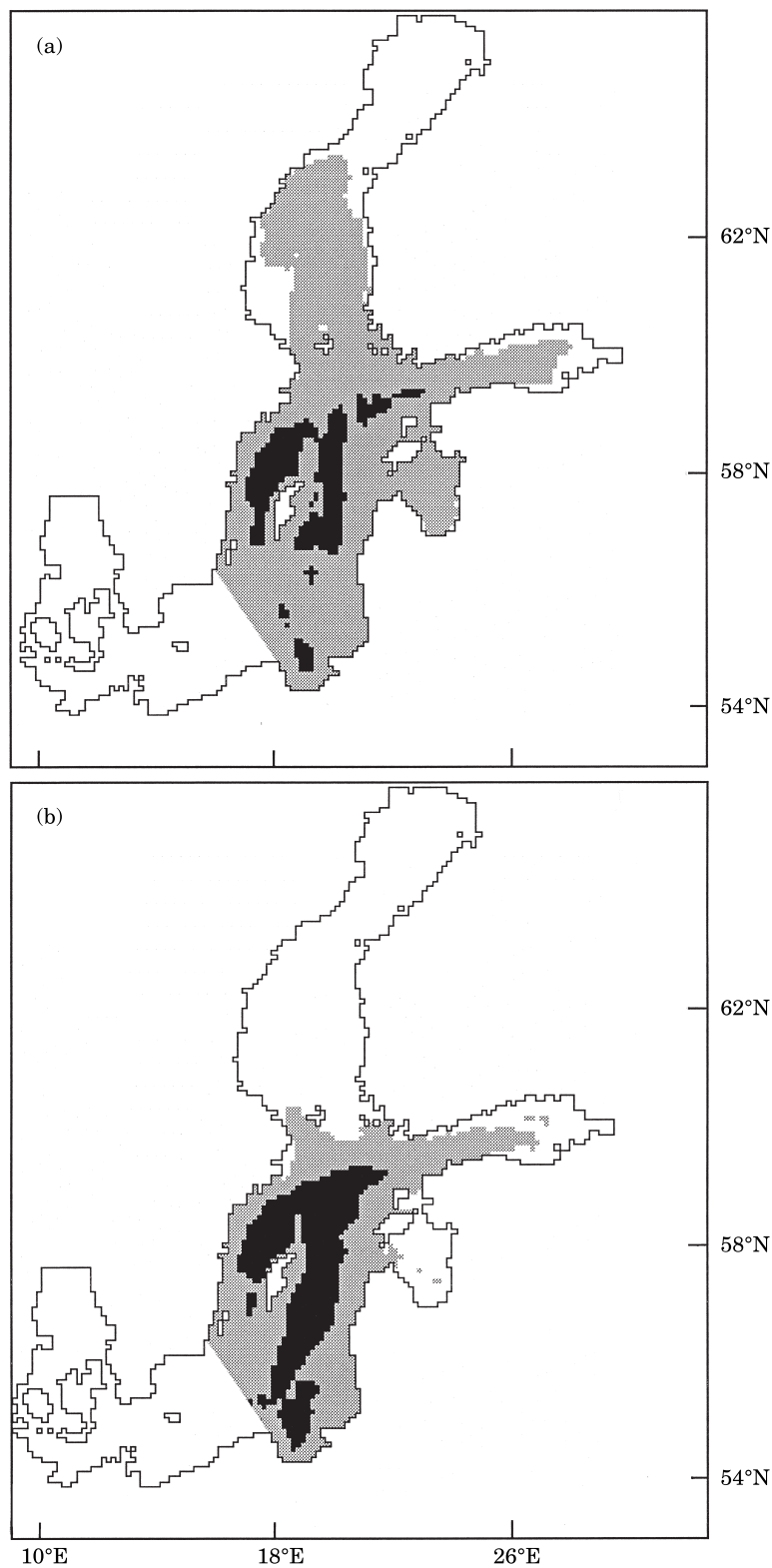


Figure 6. Distribution of reproductive area ( $\geq 6$  psu;  $\geq 2$  ml  $O_2/l$ ) of flounder with demersal eggs in the Baltic during (a) favourable (May–July 1978) and (b) unfavourable (May–July 1995) environmental conditions, respectively, based on salinity requirements in the present investigation [area inhabited by flounders with demersal eggs according to [Molander \(1925\)](#)]. Light grey, reproduction areas,  $>6$  psu salinity in the bottom layer; black, areas with  $<2$  ml  $O_2$   $l^{-1}$  in the bottom layer.

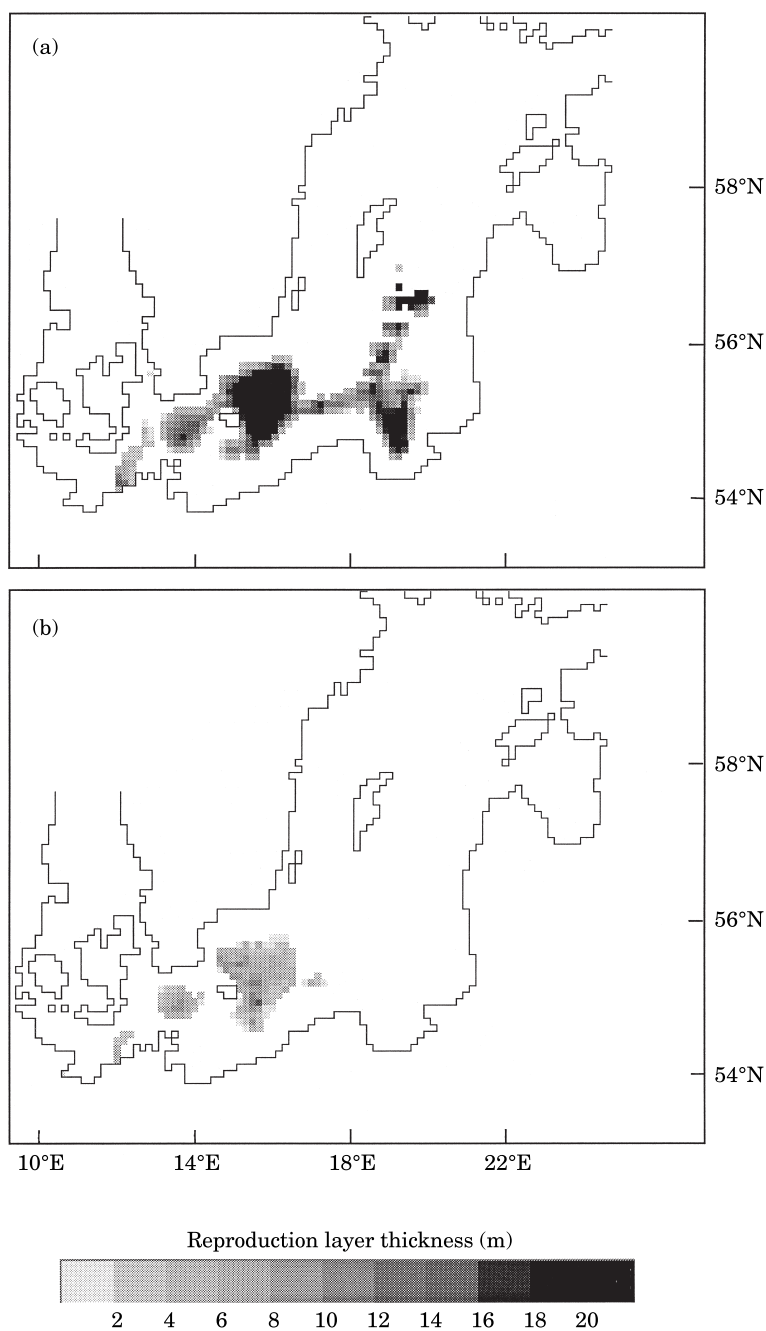


Figure 7. Distribution of reproductive volume ( $\geq 12.0$  and  $\geq 10.7$  psu respectively;  $\geq 2$  ml  $O_2/l$ ) for flounder with pelagic eggs in SD 24 and SD 25, 26, and 28 in the Baltic during (a) favourable (March–June 1972) and (b) unfavourable (March–June 1989) environmental conditions, respectively, based on salinity requirements in the present investigation.

salinity requirements for successful reproduction, are a result of long-term selection processes. Thus they are specific to populations and can be used for stock discrimination.

Stock discrimination of dab, plaice, and flounder in the Baltic has been evaluated by differences in meristic

characters as well as migration patterns. [Temming \(1989b\)](#) separated dab in the Belt Sea area (SD 22 and western part of SD 24) from dab in the Bornholm area (SD 25). This is in agreement with the present study; dab with lower salinity requirements for successful reproduction were found in central SD 24 and in SD 25 compared

to fish in SD 23 and western part of SD 24. According to Bagge and Steffensen (1989) plaice in the Baltic proper consists of one stock separated from plaice in the Belt Sea (SD 22), and flounder in the southern Baltic, consist of two populations, one in the Arkona basin (SD 24) and one in the Bornholm basin (SD 25), distinguished from flounder populations in the Belt Sea area and the Sound (SD 22 and SD 23). This is in accordance with the present investigation; one population of plaice (SD 24–28) and two flounder populations with pelagic eggs, one in SD 24 and one in SD 25, separated from flounder in SD 23. Concerning flounder with demersal eggs, analysis of tagging experiments has indicated several local populations with limited migration patterns (Bagge and Steffensen, 1989). Comparing our results with those obtained by Solemdal (1970, 1973) and Lönning and Solemdal (1972) reveals no differences in egg buoyancy [ $20.3 \pm 1.1$  psu (present investigation) compared with 19.9–20.3 psu (Solemdal, 1973; Lönning and Solemdal, 1972)] or in salinity for successful fertilisation [6 psu (present investigation) and  $\sim 6$  psu (Solemdal, 1970)] between fish caught off Gotland in SD 28 and off Tvärminne in SD 32. Hence, on the basis of salinity requirements only one population seems to be present in the area.

Throughout the years several ichthyoplankton surveys of the occurrence of fish eggs have been conducted in the Baltic deep basins. Flounder eggs have been reported from the Arkona and Bornholm basins (SD 24–25) but also from the Gotland basin (SD 28) (see Lindblom, 1973). Eggs of plaice have regularly been found in the Arkona and Bornholm basins (SD 24–25) but sometimes also in the Gdansk basin (SD 26) (Molander, 1925; Mielck and Künne, 1935; Lindblom, 1973). Dab eggs have been found in the Arkona basin (SD 24) but the highest numbers are reported from the Bornholm basin (SD 25) (Molander, 1925; Mielck and Künne, 1935; Lindblom, 1973; Temming, 1989b). For both flounder and plaice, estimations of salinity requirements for successful reproduction in the present investigation are in agreement with the occurrence of eggs according to available ichthyoplankton data. For dab, the present investigation suggests that successful reproduction may occur only occasionally in the Arkona and Bornholm basins. In the literature neutral egg buoyancy of dab in the Baltic has been reported to be obtained at  $\geq 12$  psu (Strodtmann, 1918) or at  $\geq 15$  psu (see Bagge, 1981). This is in contrast to our results ( $\geq \sim 17.8$  psu for dab caught in mainly SD 24) and suggests that there may be another population of dab, adapted to conditions occurring in the Bornholm basin (SD 25). Dab in the Bornholm area is known to spawn somewhat later than plaice and flounder, peak spawning taking place in May–July, although eggs have been found as early as March (Temming, 1989a). Thus, as fish in the present investigation were caught in February–April, dab

spawning in the Bornholm area might not have been caught. Only one female was caught in the western part of SD 25 (trawl station 55.27°N 14.44°E) showing the same SNB as fish from SD 24.

As the deep water in the Baltic is exchanged mainly during periods of saline water inflows from the North Sea, conditions for reproduction vary greatly. Salinity in the deepest parts of the Baltic deep basins vary between  $\sim 14$ –20 psu in the Arkona basin (SD 24),  $\sim 13$ –22 psu in the Bornholm basin (SD 25), and between  $\sim 10$ –14 psu in the Gdansk and Gotland basins (SD 26 and SD 28) (Fonselius, 1962; Matthäus, 1993; Mattäus *et al.*, 1994), i.e. opportunities for eggs to achieve neutral buoyancy and to remain above layers with poor oxygen conditions vary. Favourable spawning conditions occurred after inflow events in the 1950s, 1960s, and late 1970s whereas poor conditions prevailed during e.g. the 1980s up to early 1990s due to the longest known stagnation period without inflows (see e.g. Franck *et al.*, 1994; Matthäus and Lass, 1995). As shown in Figures 2, 4, 6, and 7, the extension of habitats suitable for reproduction and thus supporting the stock vary greatly, affecting both stock abundance and species distribution not only for dab, plaice and flounder with pelagic eggs but also for flounder with demersal eggs.

There is only limited information about the potential effects of salinity on stock development of Baltic flatfish populations. Ojaveer *et al.* (1985) reported an increase in the stock of flounder in the Gulf of Finland (SD 32) during the 1970s in connection with an increase in salinity, followed by a decrease in stock size in the early 1980s as salinity decreased. However, the reason for the flatfish stock breakdown in the southern Baltic during the 1930s could, apart from overfishing, be a deterioration in conditions suitable for reproduction. Whereas total landings of plaice and flounder have recovered, those of dab have remained low (Temming, 1989a). Both plaice and flounder recovered during the 1950s at the same time as major saline water inflows occurred (see Franck *et al.*, 1987) but dab did not. According to present investigation dab display the highest salinity requirements for successful reproduction of the Pleuronectids in the Baltic. Hence, under deteriorating conditions dab would be the first species to suffer from reproductive failure affecting recruitment and stock development. Increased nitrogen and phosphorus input into the Baltic Sea during the 20th century has caused increased primary production accompanied by increased oxygen depletion in the deep areas, i.e. shorter periods with favourable spawning conditions. In agreement with generally poorer conditions nowadays, ichthyoplankton investigations in the Baltic deep basins during the 1960s and 1970s show much lower egg abundance compared to early in the 20th century not only for dab but also for plaice and flounder (Kändler, 1949; Lindblom, 1973; Temming, 1989a).



## Acknowledgements

We thank the crew at RV "Argos" as well as two anonymous referees. The investigation was supported by the Gunvorand Josef Aneers Foundation and the Swedish Natural Science Research Council.

## References

- Bagge, O. 1981. Demersal fishes. In *The Baltic Sea*, pp. 311–333. Ed. by A. Voipio. Elsevier Oceanographic Series no. 30. Elsevier Scientific Company, Amsterdam.
- Bagge, O., and Steffensen, E. 1989. Stock identification of demersal fish in the Baltic. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer*, 190: 3–16.
- Coombs, S. H. 1981. A density-gradient column for determining the specific gravity of fish eggs, with particular reference to eggs from the mackerel *Scomber scombrus*. *Marine Biology*, 63: 101–106.
- Dixon, W. J., and Massey, F. J. 1983. *Introduction to statistical analysis*. McGraw-Hill Inc., Tokyo. 678 pp.
- Fonselius, S. H. 1962. Hydrography of the Baltic deep basins. Fishery Board of Sweden, Series Hydrography, 13. 41 pp.
- Franck, H., Matthäus, W., and Sammler, R. 1987. Major Baltic inflows during this century. *Beiträge zur Meereskunde*, Berlin, 56: 81–82.
- Hohendorf, K. 1968. Zur Schwebfähigkeit pelagischer Fischeier in der Ostsee. *Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung*, 19: 181–193.
- Holliday, F. G. T. 1965. Osmoregulation in marine teleost eggs and larvae. *California Cooperative Oceanic Fisheries Investigations Reports*, 10: 89–95.
- Howell, B. R., Child, A. R., and Houghton, R. G. 1991. Fertilization rate in a natural population of the common sole (*Solea solea* L.). *ICES Journal of Marine Science*, 48: 53–59.
- Kändler, R. 1944. Untersuchungen über den Ostseedorsch während der Forschungsfahrten mit dem RFD "Poseidon" in den Jahren 1925–1938. *Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung (Neue Folge)*, 11: 137–245.
- Kändler, R. 1949. Die Häufigkeit pelagischer Fischeier in der Ostsee als Maßstab für die Zu- und Abnahme der Fischbestände. *Kieler Meeresforschungen*, 6: 73–89.
- Kjesbu, O. S., Kryvi, H., Sundby, S., and Solemdal, P. 1992. Buoyancy variations in eggs of Atlantic cod (*Gadus morhua*) in relation to chorion thickness and egg size: theory and observations. *Journal of Fish Biology*, 41: 581–599.
- Lindblom, R. 1973. Abundance and horizontal distribution of pelagic fish eggs and larvae in the Baltic Sea 1967–1971. *Meddelande från Havsfiskelaboratoriet, Lysekil*, 140. 33 pp.
- Löning, S., and Solemdal, P. 1972. The relation between thickness of chorion and specific gravity of eggs from Norwegian and Baltic flatfish populations. *Fiskeri-Direktoratet Skrifter Serie HavUndersøgelser*, 16: 77–88.
- Matthäus, W. 1993. Major inflows of highly saline water into the Baltic Sea – a review. *ICES CM 1993/C*: 52, 10 pp.
- Matthäus, W., and Lass, H. U. 1995. The recent salt inflow into the Baltic Sea. *Journal of Physical Oceanography*, 25: 280–286.
- Matthäus, W., Nehring, D., and Nausch, G. 1994. Effects of the inflows of salt-rich water during 1993 and early 1994 in the central Baltic Sea. *ICES CM 1994/Q*: 3, 9 pp.
- Marx, W., and Henschel, J. 1941. Die Befruchtung und Entwicklung von Plattfischeiern in verdünntem Nordwasser im Vergleich zu den Befunden in der freien Ostsee. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 2: 226–243.
- Mielck, W., and Künne, C. 1935. Fischbrut und Planktonuntersuchungen auf dem RFD Poseidon in der Ostsee 1931. *Wissenschaftliche Meeresuntersuchungen (Abt. Helgoland)*, 19: 1–120.
- Molander, A. R. 1925. Undersökningar över Rödspotta (*Pleuronectes platessa*), Flundra (*Pleuronectes flesus*) och Sandskädda (*Pleuronectes limanda*) i södra Östersjön. *Svenska Hydrografisk-Biologiska Kommissionens Skrifter*, 1. 38 pp.
- Morisawa, M., and Suzuki, K. 1980. Osmolality and potassium ion: their role in initiation of sperm motility in teleosts. *Science*, 210: 1145–1146.
- Nissling, A., Kryvi, H., and Vallin, L. 1994. Variation of egg buoyancy of Baltic cod *Gadus morhua* and its implications for egg survival in prevailing conditions in the Baltic Sea. *Marine Ecology Progress Series*, 110: 67–74.
- Nissling, A., and Westin, L. 1991a. Egg buoyancy of Baltic cod (*Gadus morhua*) and its implications for cod stock fluctuations in the Baltic. *Marine Biology*, 111: 33–35.
- Nissling, A., and Westin, L. 1991b. Egg mortality and hatching rate of Baltic cod (*Gadus morhua*) in different salinities. *Marine Biology*, 111: 29–32.
- Nissling, A., and Westin, L. 1997. Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. *Marine Ecology Progress Series*, 152: 261–271.
- Ojaveer, E., Kaleis, M., Aps, R., Lablaika, I., and Vitins, M. 1985. The impact of recent environmental changes on the main commercial fish stocks in the Gulf of Finland. *Finnish Fisheries Research*, 6: 1–14.
- Sokolov, A., Andrejev, O., Wulff, F., and Rodriguez Medina, M. 1997. *The Data Assimilation System for Data Analysis in the Baltic Sea*. Systems Ecology Contributions No. 3. Department of Systems ecology, Stockholm University, Stockholm.
- Solemdal, P. 1967. The effect of salinity on buoyancy, size and development of flounder eggs. *Sarsia*, 29: 431–442.
- Solemdal, P. 1970. The reproductive adaptation of marine teleosts to water of low salinity. *ICES CM 1970/F*: 30, 3 pp.
- Solemdal, P. 1971. Prespawning flounders transferred to different salinities and the effects on their eggs. *Vie et Milieu (suppl.)*, 22: 409–423.
- Solemdal, P. 1973. Transfer of Baltic flatfish to a marine environment and the long term effects on reproduction. *Oikos (suppl.)*, 15: 268–276.
- Stoss, J. 1983. Fish gamete preservation and spermatozoan physiology. In *Fish physiology*, vol. IXB, pp. 305–350. Ed. by W. S. Hoar, D. J. Randall, and E. M. Donaldson. Academic Press, New York.
- Strodtmann, S. 1918. Weitere Untersuchungen über Ostseefische, III. *Wissenschaftliche Meeresuntersuchungen (Abt. Helgoland)*, 14: 31–95.
- Temming, A. 1989a. Migration and mixing of dab (*Limanda limanda*) in the Baltic. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer*, 190: 25–38.
- Temming, A. 1989b. Long-term changes in stock abundance of the common dab (*Limanda limanda*) in the Baltic proper. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer*, 190: 39–50.
- Thorsen, A., Kjesbu, O. S., Fyhn, H. J., and Solemdal, P. 1996. Physiological mechanisms of egg buoyancy in brackish water cod (*Gadus morhua* L.). *Journal of Fish Biology*, 48: 457–477.
- Vallin, L., and Nissling, A. 2000. Maternal effects on egg size and egg buoyancy of Baltic cod, *Gadus morhua* – implications

- for stock structure effects on recruitment. Fisheries Research, 49: 21–37.
- Voipio, A. (ed.) 1981. The Baltic Sea. Elsevier Oceanographic Series no. 30. Elsevier Scientific Company, Amsterdam. 418 pp.
- Westernhagen, H. v. 1970. Erbrutung der von Dorsch (*Gadus morhua*), Flunder (*Pleuronectes flesus*), und Scholle (*Pleuronectes platessa*) unter kombinierten Temperatur- und Salzgehaltsbedingungen. Helgoländer Wissenschaftliche Meeresuntersuchungen, 21: 21–102.
- Wieland, K., Waller, U., and Schnack, D. 1994. Development of Baltic cod eggs at different levels of temperature and oxygen content. Dana, 10: 163–177.