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THE VIVIPAROUS BLENNY AS AN INDICATOR OF EFFECTS OF TOXIC SUBSTANCES

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INTRODUCTION

This is the final report from the project "Viviparous blenny as an indicator of effects of toxic substances" designed to develop a system of environmental monitoring where the viviparous blenny, or eel-pout, (*Zoarces viviparus* L.) is used as indicator species for occurrence of toxic substances in the sea and for ecological effects of such substances. The system should be possible to use both within programmes concerning monitoring of emissions and receiving waters as well as in long-term monitoring of reference areas. Several single species systems have been developed in order to enable laboratory measurements of biological effects of toxic substances. Reactions observed are, however, frequently difficult to transfer to ecologically relevant disorders in nature. A single species system for programmes concerning control of emissions and receiving waters should include not only measurement of effects on the individual level in the laboratory and the field, but also monitoring of population variations as well as, if the relevant substances are persistent and bioaccumulating, chemical analyses. If the species chosen is a fish, it should have the following properties:

- 1. A **stationary mode of living** throughout its entire life cycle. This is essential for linking observations to pollution in the controlled area.
- 2. Sufficiently **large in size** to provide samples large enough for individual chemical and physiological analyses.
- 3. **Easy to catch**, also as fry.
- 4. **Rich abundance**, preferably within a **large area of dispersion**.
- 5. **Long life**, which makes it possible to integrate effects through time and to investigate the importance of long-term exposure.
- 6. The possibility of **determining age** is required for analysis of dose/effect and for studies of the population's age distribution, which may indicate disorders in reproduction or increased mortality.
- 7. **Suitability for monitoring of emissions**, which implies that it should be possible to register ecologically important effects of emission not only under controlled laboratory conditions but also in the field.

Jacobsson et al. (1986) demonstrated that the viviparous blenny has the above-mentioned characteristics. The authors base this statement on earlier knowledge as regards the six first requirements and on an experiment described in their paper with regard to the seventh. Several freshwater species also have the required properties but hardly any other marine species present in Swedish waters. This implies that the coast of western Sweden lacks good alternative indicator species; the national monitoring of concentrations of harmful substances makes use of the non-stationary flounder. Also in relation to our freshwater fishes, the blenny has an important advantage: It gives birth to live fry after a long gestation period (4—6 months). In this way, a general problem is eliminated in studies of reproduction parameters in fish, namely the difficulty to identify brood origin and measure reproduction success among individuals.

In the above-mentioned experiment, use was made of the blenny´s special fry biology. For one month, pregnant females were exposed to low concentrations of waste-water emanating from a forest industry, after which deteriorated growth of fry could be established. At higher concentrations mortality was high; female survival was, however, not influenced. The fry were thus, in the same way as in "normal" fish species, more susceptible than adults. Fry which were not exposed to waste-water showed no mortality whatsoever after the period immediately following hatching. This relationship is of great value since the large and strongly varying mortality occurring during the first months of life in fish species with free-swimming fry causes considerable difficulties in both field and laboratory studies. The fry of viviparous blenny are large (up to 55 mm long) at birth and have thus passed the stages normally considered to be decisive for stock dimensioning among fish.

The properties of the blenny, particularly its reproduction biology, may justify utilization of the species also on the Swedish east coast, which lies within the dispersion area of the species. The behaviour of the species and its reproduction biology along the east coast have therefore been investigated and are discussed in this report.

The intention has been to develop a "blenny system" for registering effects of toxic substances on the reproductive ability, mortality and growth, as well as monitoring concentrations of persistent and bioaccumulating substances. A programme for control of receiving waters with parallel investigations in reference areas, may have the following design:

- ◆ 1. Test fishing for monitoring of relative abundance of adult fish.
- ◆ 2. Age distribution in the test fishing catches.
- ◆ 3. Growth rate of the fish caught.
- ◆ 4. Number of live and dead fry in females caught in test fishing.
- ◆ 5. Size, i.e. growth rate, of live fry during pregnancy.
- ◆ 6. When relevant, analysis of persistent and bioaccumulating substances.

In emission monitoring programmes and characterization of waste-water, as well as in short-term surveys of environmental affects, the variables in 4, 5 and 6 will be of greatest relevance. Since the biology and reactions of blenny to pollution are relatively little known, a programme of stated character requires several pre-studies. The following have been conducted within the project:

- ◆ 1. Seasonal migrations.
- ◆ 2. Methods for monitoring of abundance.
- ◆ 3. Fecundity, growth and mortality of fry in unpolluted areas on the west and east coasts.
- ◆ 4. Growth and mortality of fry in polluted areas.
- ◆ 5. Experiments into toxic effects on reproduction.
- ◆ 6. Concentration of persistent and bioaccumulating substances in adult fish.

AREAS OF INVESTIGATION

The investigations have been conducted in areas without emissions of harmful substances and also in waters receiving emissions from industries and municipalities. Unpolluted areas studied are Fjällbacka, Ringhals, northern Kalmarsund, Kvädöfjärden, Öregrundsgrepen and Holmöarna (Figure 1). At Fjällbacka, Kvädöfjärden, and Holmöarna, fish have been collected for the national contaminant monitoring programme annually since 1980. Since 1988, preliminary studies have also been made in these areas with the intention of widening the programme with integrated ecological and physiological aspects. At Fjällbacka, viviparous blenny is utilized for individual studies conducted within the programme. The control programme for the Ringhals nuclear power station, including test fishing with small eel fyke nets giving good catches of blenny, has been conducted since 1976 in the heated discharge area and in the unaffected Vendelsöfjorden (Figure 2). The project has also included test fishing in Båtfjorden which is just to the south of the power station and not exposed to direct influence of heated water. At Kalmarsund and Öregrundsgrepen, information on abundance variations of the species has been obtained within the control programmes for the Oskarshamn and Forsmark nuclear power stations.

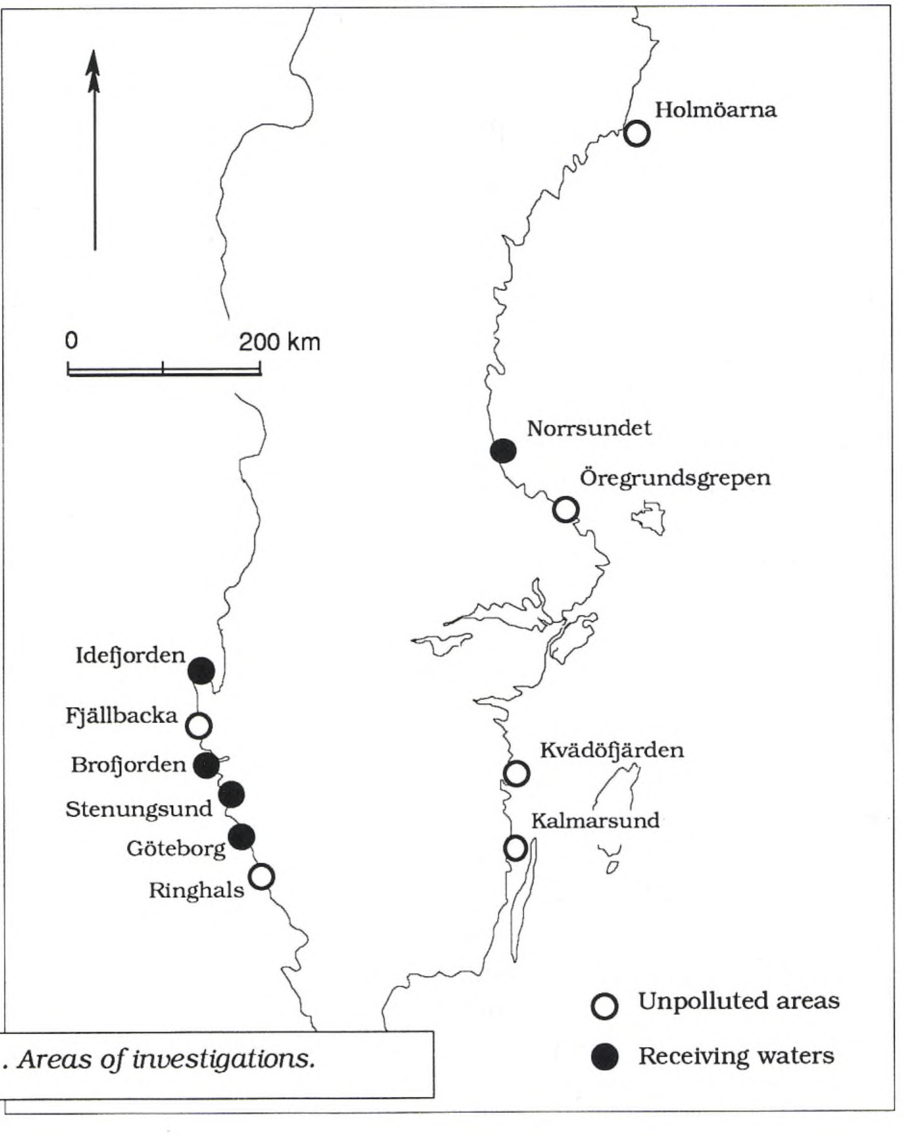

***Figure 1**. Areas of investigations.*

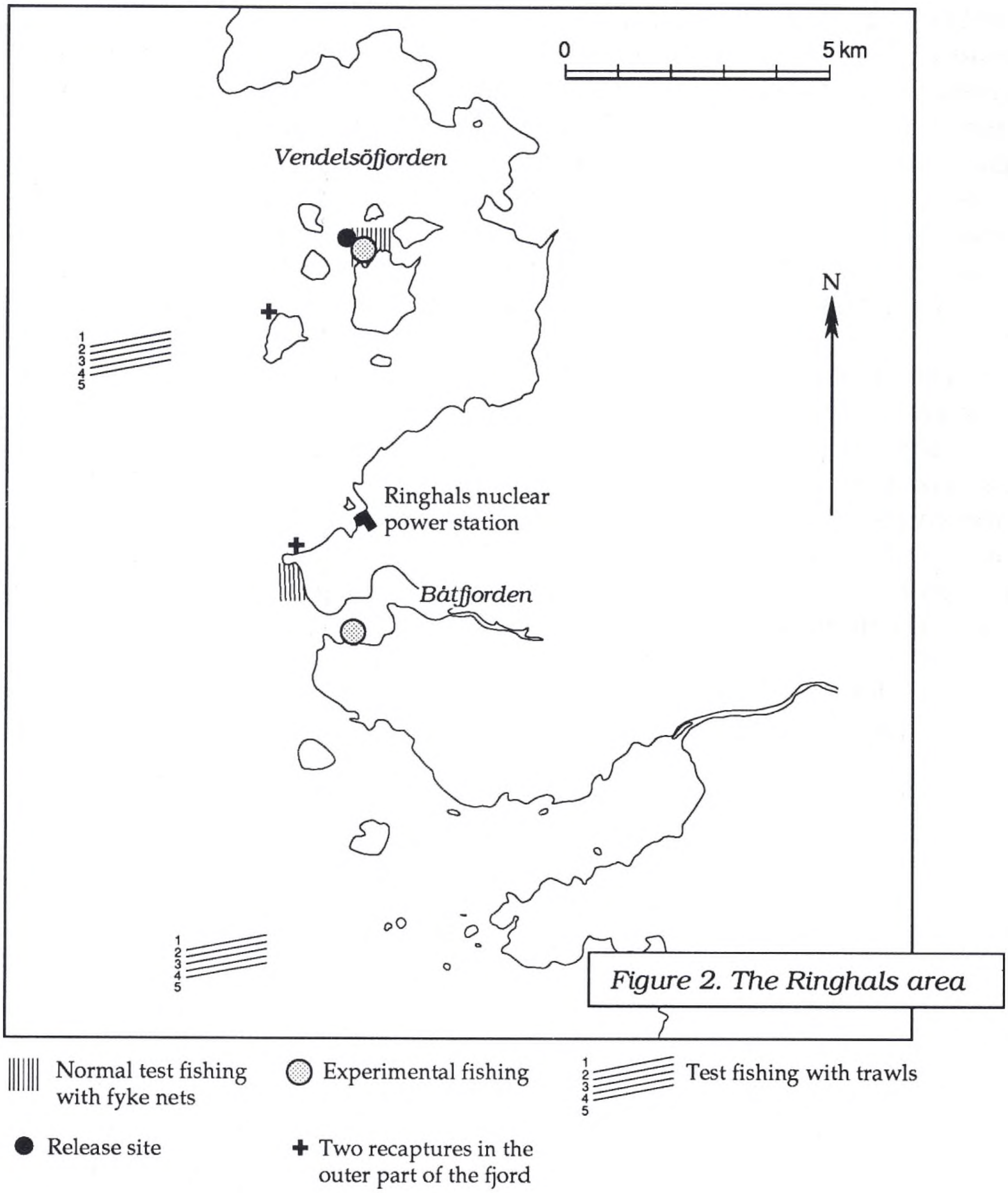

Figure 2. The Ringhals area

Receiving waters for testing effects on reproduction were chosen to be Idefjorden, Brofjorden, Stenungsund and Göteborg at the west coast and Norrsundet in the Bothnian Sea (Fig. 1). Idefjorden received muncipal discharges from the town Halden and from a bleached pulp mill. Brofjorden receives discharges from an oil refinery. At Stenungsund, there are four petrochemical factories which probably are responsible for severe reduction of young plaice during the last ten years (Jacobsson and Neuman, 1991). At Göteborg, material was collected just outside the port area. This area is exposed to a complex influence including the River Göta Älv and the neighbouring municipal sewage works. At Norrsundet, there is a bleached pulp mill at which the receiving waters have shown effects on fish at all levels from cell to community. Close to the discharge point, the occurrence of fry of several species has been abnormally low (Neuman and Karås, 1988; Karås et al., 1991).

SEASONAL MIGRATIONS

Genetical investigations (Schmidt, 1917; Frydenberg et al., 1973; Christiansen et al., 1976) have shown that blenny is to be regarded as stationary throughout its entire life cycle. The occurrence of short movements, e.g., seasonal migrations, within a larger home area cannot, however, be excluded. It is important to survey movements of this kind, particularly in studies of receiving waters. Consequently, material has been collected from test fishings with small eel fyke nets off Ringhals, in Kvädöfjärden and at Holmöarna, and from tagging at Ringhals.

The seasonal migrations of fish are mainly controlled by their physiologically conditioned temperature preferences. Those of the blenny can be illustrated by means of catches in April and August at depths of 2—4 m in the proximity of the cooling water discharge at Ringhals, where there is a wide spread over the temperature scale. The largest catches were made in the 4—12°C range, whereas catches above 14°C were small (Figure 3). The species is thus a cold-water species, which may be expected to migrate down to colder water during the summer.

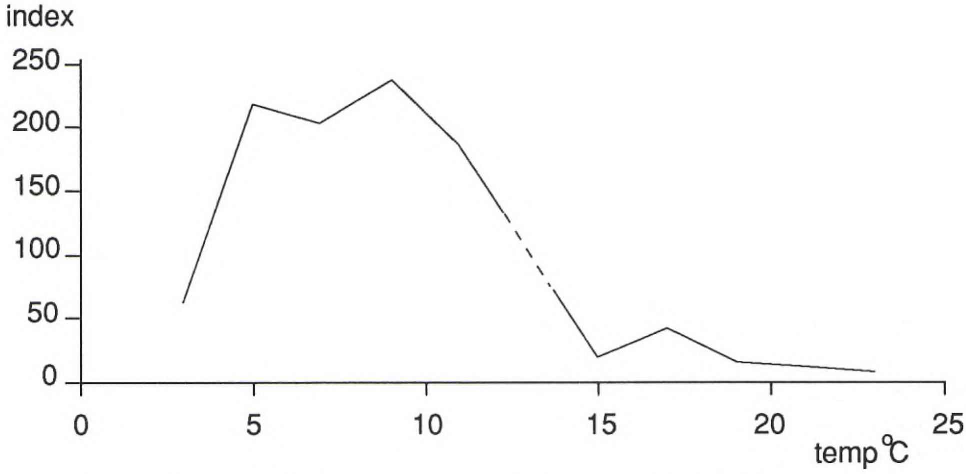

Figure 3. *Influence of temperature of blenny catches off Ringhals, 1976—1987. The mean value of the catch per effort within 2°C temperature intervals has been related to the corresponding mean value for the entire catch (=100).*

Seasonal migrations were studied in Vendelsöfjorden off Ringhals with four eel fyke nets at three depths: 0.5, 3.5 and 7.0 m. Fishings were done on three consecutive days per month during the period from March 1985 until January 1986. The total catches at 0—7 m were:

Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
34	11	32	5	1	26	2	13	17	20	35

During the summer, with the exception of August, the catches were very small, suggesting that most individuals had moved to deeper water. Blenny spawn in August which may possibly explain the good catches at that time, mainly at 3.5 m. During November—January, 60—70% were caught at 0.5 m, where the species was totally absent in June and July. In this area, extensive experimental trawling has been done at depths of 20 m in September since 1983 (Figure 2). Only one blenny has been caught, which suggests that they do not migrate down to this depth.

Since collection of females for examination of fry took place in October—November, the depth distribution was investigated in October 1986 by means of fishing at depths of 1, 2, 3, 4 and 5 m in Vendelsöfjorden and Båtfjorden (Fig. 2). Good catches were obtained at all depths but the best catches were at 1 and 2 m.

Movements of the blenny were also studied by means of tagging in Vendelsöfjorden, where direct exposure to the open sea could be expected to result in greater migration tendency than in more protected areas. The "Floy tag", a numbered tag attached beneath the dorsal fin, was used. Fish were caught in shallow water (Fig. 2) and releases were made in the same area where also recaptured fish were released. In order to avoid infections, taggings were only done during the cold part of the year. A total of 223 fish were tagged in May 1985, 199 in January 1986 and in October and April 1986—87 and 1987—88, 185 and 1410 fish respectively, totalling 2017.

Despite an estimated loss of up to 30% of the tags soon after release, it was possible to recapture 600 individuals at least once, of which 487 during the last tagging season. Since several fish were caught on a number of occasions — one fish returned a total of seven times to the same station — the total number of recaptures was 907. Altogether 403 fish were recaptured once, 123 fish were caught twice, 52 were caught three times, 14 four times, 3 five times, 4 six times and 1 fish seven times.

No recapture was reported outside the fjord and only two from its outer areas despite the eel fishermen in the area being well informed about the experiments. All other fish were captured in our own test fishing programme within the limited release area, which suggests a very stationary way of living. With regard to the low intensity of test fishing, the recapture percentage was very high.

Together with the test fishing reported earlier, the tagging suggests that this species migrates down from shallow areas during the summer. There were few recaptures in the test fishing during the summer, mainly August, but they were more numerous during the "winter", mainly October and April (Table 1). The difference can only partly be explained by larger fishing input during the cold part of the year. Many fish return to the release area after one or more summers, which they probably spend in deeper water. The investigation also provides some information on the longevity of the species. No fish has been caught more than three years after tagging and age determinations have not revealed individuals older than four years.

Table 1. *Recaptures during different seasons different lengths of time after tagging.*

Year	1st	2nd	3rd	4th
Winter	704	99	13	3
Summer	81	5	2	0

Fishing with small eel fyke nets was done during the autumn of 1989 at Holmöarna and in Kvädöfjärden in order to examine catch possibilities and any seasonal migration at this time. At Holmöarna, the fyke nets were placed out at fixed stations at 3, 7—8 and 21 m depth; eight pairs of fyke nets at each depth. The nets were emptied once a week during weeks 38—47. At depths of 3 m only one fish was caught. Poor catches were also given by one of the two stations at 7—8 m. Catches at the other station and the deep station are shown in Table 2 together with results from Kvädöfjärden. The period then concerned was weeks 38—44 in the depth range 2—8, 5—8 and 13—16 m.

Table 2. *Catches (number/fyke net and night) during the autumn of 1989.*

	week								
	38	*39*	*41*	*42*	*43*	*44*	*45*	*46*	*47*
Holmöarna									
7 m	0.07	0.58	0.14	0.17	0.08	0.06	0.23	0.06	0.04
21 m	0.16	0.52	0.35	0.23	0.14	0.09	0.36	0.69	0.15
Kvädöfjärden									
2—8 m	0.18	0.45	0.75	0.28	0.19	0.50			
5—8 m	0.30	0.50	0.77	1.01	0.33	0.82			
13—16 m	0.23	0.09	0.06	0.10	0.25	0.09			

The deepest site gave the best catches at Holmöarna but the poorest catches in Kvädöfjärden. It was not possible to distinguish any seasonal migration in this material. During weeks 43—47, fishing was done at several other stations in addition to those at the fixed stations in order to find good sites. Good catches were obtained at 6—12 m at Holmöarna and also at 3—4 m during the final week. This may possibly indicate that the species was moving into shallow water late in the season. In Kvädöfjärden, the more intensive fishing was done only at 5—8 and 5—15 m; the shallow sites clearly gave the best catches.

Smaller inventories were made during weeks 44—48 in 1989 at Kalmarsund at 2—20 m and at Öregrundsgrepen at 3—15 m. Occasional fishes were found at the deepest stations. The best catches were made at 6—8 m at Kalmarsund and at 4—5 m at Öregrundsgrepen. In the same way as at Holmöarna, the catches increased at Kalmarsund in shallow water (2—3 m) towards the end of the period. Kalmarsund gave slightly better catches than Kvädöfjärden and Holmöarna and clearly better than Öregrundsgrepen. There does not appear to be any clear dependency in abundance with regard to latitude or salinity.

The fishing inputs along the east coast have shown that the species can easily be caught during the autumn in eel fyke nets. Here it appears to be more common in deeper water than on the west coast; in the western Gulf of Finland researchers have caught even young-of-the-year at depths of 25—40 m (Kristofferson and Oikari, 1975). However, the best catches were obtained at 3—8 m. The investigations during 1989 do not indicate any particular difference in population abundance between unpolluted areas on the west and east coasts. However, our long-term monitoring in Kalmarsund shows that population abundance increased strongly during 1986—89 and, thus, the abundance was probably unusually high along the east coast in 1989 (Neuman and Andersson, 1990). The development in Vendelsöfjorden suggests that a corresponding development did not take place along the west coast (Jacobsson, 1991).

MONITORING OF ABUNDANCE

Since the mid-1970's we have used small eel fyke nets to monitor abundance as part of the control of recipient waters on the west coast. Neuman (1983, 1988) demonstrated that effects of cooling water discharge on the abundance of blenny could be established with fairly small inputs using fyke nets of this kind. A population monitoring designed for viviparous blenny requires, however, further knowledge as regards, e.g., fishing intensity. If possible the monitoring should be conducted during October when the females carry fry which are sufficiently large to enable the easy registration of any growth disorders and when the weather conditions are still not too severe. During weeks 40—42 in 1987—1989, test fishing was done at Ringhals both in the open Vendelsöfjorden and also in the more enclosed Båtfjorden (Figure 2).

Under "seasonal migrations" it was noted that the best catches in October were made at depths of 1 and 2 m. Since catches at 1 m may possibly vary widely as a result of changes in water level and waves, the test fishing was done at 2 m. The fyke nets were grouped within each area at five stations with ten nets in a row, all opening in the same direction. The distance between the stations was maximally 30 m. The nets were used for eight days and were emptied daily. This input is more intensive than what may be considered reasonable for routine monitoring. In this way, it was possible to examine whether tendencies for over-fishing were present which would imply that the observations could not be regarded as independent. A major input also makes it possible to find a suitable level for future inputs by means of reductions in the data obtained.

The catches did not decrease with time, which must be interpreted as not even intensive inputs leading to "over-fishing". Catches on different days may thus be regarded as independent even within the fairly enclosed Båtfjorden.

Mean catches per station and confidence intervals (95%) for the two areas in the various years are presented in Figure 4A. The range is relatively small. The spread between days for individual stations and between

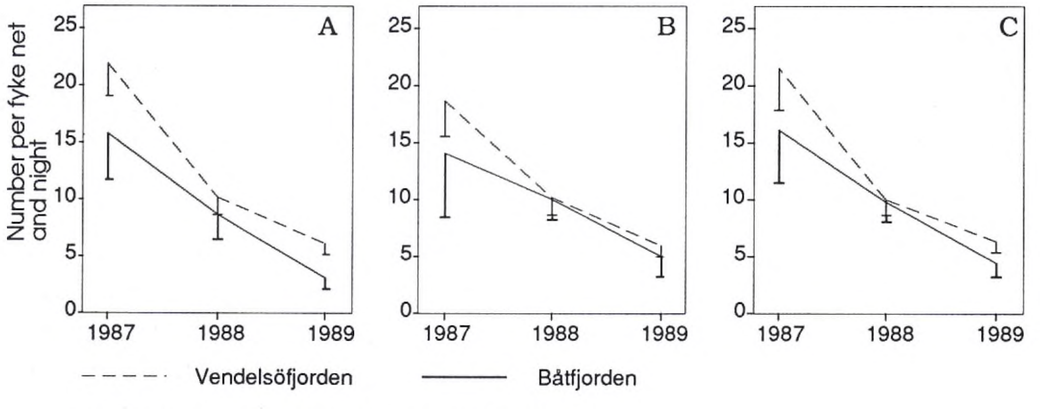

Figure 4. *Intensive fishing at Ringhals*
A. Entire material: 8 days and 50 fyke nets
B. The four first days and 50 fyke nets
C. The six first days and 25 fyke nets.

stations on individual days are approximately of similar magnitude, with a few exceptions. Only one station in Vendelsöfjorden diverges clearly from the others and also shows different spreads in different years.

The differences between years and areas shown in Figure 4A were tested with the non-parametric H-test and were found significant. For conducting a parametric ANOVAtest, the variance was stabilized by logarithmation of the material; the station mean values otherwise do not have the same variance within the area. Transformation by logarithmation also gives a material that is approximately normally distributed (Kolmogorov-Smirnov test, Conover, 1971). A two-way analysis of variance gives significant differences between years and between areas.

In order to study ways of restricting the inputs, the last fishing days have been eliminated from the material. As shown by Figures 4A and B, the catch remained similar even when the number of days was halved to four fishing days. The H-test also still shows a significant difference between years and areas. The conclusion of this analysis is that six days would have been more than sufficient in the present case. While general recommendations can be made as regards the number of fishing days, the amount of gear or stations must be adapted to local conditions and to the need of obtaining fish for different analyses. Figure 4C shows six fishing occasions with alternate observations (day catches per station) removed. The conclusion will be that in the investigated area the abundance monitoring can be done with 25 fyke nets if a total of six days are fished.

FECUNDITY, GROWTH AND MORTALITY OF FRY IN UNPOLLUTED AREAS

Occurrence of fry in females was investigated in late October until mid-November. The fry have then lived in the females for about two months after hatching. Three distinct size groups were distinguished: "Normal", "small" and "early-dead". In all areas, the first category made up most of the fry and consisted of living fry with lengths of 30—50 mm. The early-dead fry died soon after hatching but nonetheless are preserved in the ovarian fluid; they are <15 mm long. Among the "small" fry, there are both those that die in a late stage ("late-dead") and also those that are alive but with inhibited growth. These groups can only be distinguished in fresh material. The length of the "small" fry varied between 20 and 30 mm. The number per female was registered separately for all three categories and for "normal" and "small" fry the registration also included total weight and thus mean weight.

A primary measure in reproduction biology is fecundity, i.e., the number of eggs per female. Since also dead fry remain in the ovarium it is possible to use the number of fry as a measure of fecundity. The relative fecundity, i.e., the number of fry in relation to the somatic weight of the female (without reproductive organs and digestive apparatus) ha been calculated for material from Vendelsöfjorden in 1987, from Fjällbacka, Kvädöfjärden and Holmöarna in 1989 and from Fjällbacka in 1990. There is a significant

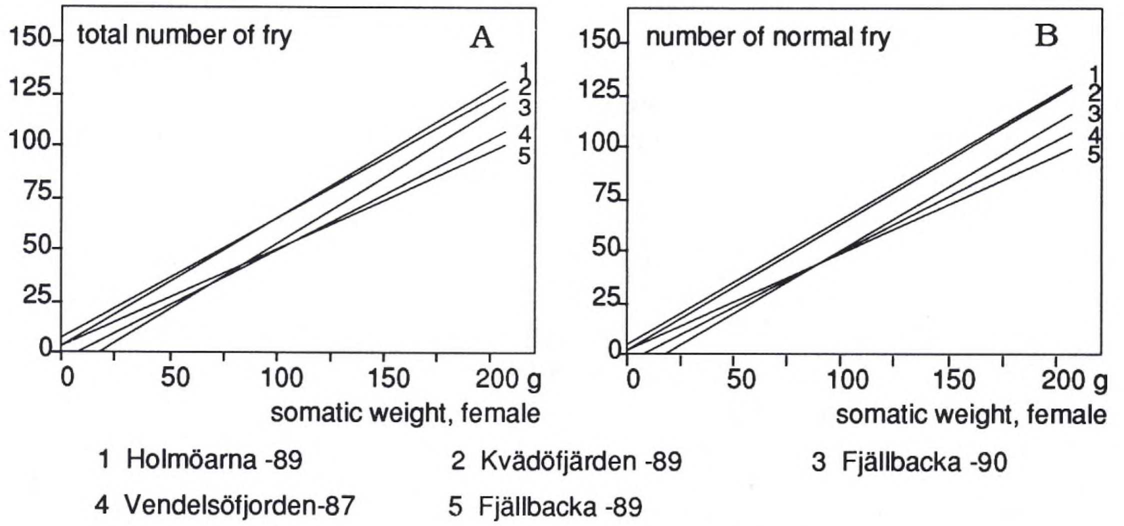

Figure 5. *Number of fry in relation to the weight of the female.*

dependence on size; the female´s weight explains 50—75% of the variation in number of fry (Figure 5A). Fecundity was slightly higher at the east coast sites than on the west coast, but the material from Fjällbacka shows that the difference between years in one area can be just as large as the difference between areas.

The influence of female size on production of "normal" fry has been investigated by relating number, total weight and mean weight of such fry to the female´s somatic weight. The number of fry and their total weight increase with length in about the same way as fecundity (Fig. 5B) but their mean weight is not influenced. As regards production of normal fry, Holmöarna and Kvädöfjärden were slightly higher than Fjällbacka and Vendelsöfjorden.

Table 3 gives a comparison of mean weights in different weeks for all areas investigated in 1989, thus also including polluted areas. The differences between areas should be regarded as small; the size at Holmöarna does not diverge despite the low salinity and the northern position. The comparison suggests that the species spawns approximately simultaneously around the entire coast of Sweden and that growth rate does not vary particularly

Table 3. *The mean weight of normal fry in 1989. Mean weight (g) was calculated as the mean of the mean values of the individual females.*

Week	*Fjällbacka -88*	*-89*	*-90*	*Ide-fjorden*	*Bro-fjorden*	*Stenung-sund*	*Göte-borg*	*Kvädö-fjärden*	*Holm-öarna*
38								0.17	
39									0.17
41									0.21
42								0.16	0.19
43		0.20					0.18	0.23	
44								0.21	0.25
45	0.24			0.27				0.29	
46			0.20			0.28	0.32		0.24
47				0.28		0.33			0.31
49							0.37		
50					0.34				

between different coastal areas. For Fjällbacka, the values for 1988 and 1990 have also been included in the table. They indicate that differences may occur between years; the mean weight of 0.2 g was reached in 1989 in week 43 but not until week 46 in 1990.

Analyses of fresh material, which is a condition for assessment of whether fry are alive or not, were made in the reference area in Fjällbacka in 1988 and in Vendelsöfjorden in several years, the last being in 1987. In this material, not one single fry was found to have died in a late stage. On frozen material, which dominates from the reference areas, the analysis must be restricted to occurrence of early-dead and small fry (Table 4).

Table 4. *Occurrence of early-dead and small fry in the unpolluted areas.*

Area	*n*	*% of females with* early-dead	*small*	*% of the total no of fry* early-dead	*small*
Vendelsöfjorden -87	100	12	4	1.1	0.3
Fjällbacka -88	145	49	26	3.2	1.2
-"- -89	95	45	26	1.9	0.7
-"- -90	59	58	25	3.5	0.9
Kvädöfjärden -89	114	7	13	0.5	0.4
Holmöarna -89	219	–	19	–	3.0

n = number of females

The occurrence of early-dead fry was low in Vendelsöfjorden and Kvädöfjärden whereas every second female at Fjällbacka had such fry. No registration was done at Holmöarna. Also the share of females with small fry varied strongly between areas with the lowest share in Vendelsöfjorden and the highest at Fjällbacka. The share of small fry of the total number of fry was low in all areas; Holmöarna had the highest share.

GROWTH AND MORTALITY OF FRY IN POLLUTED AREAS

As mentioned earlier, growth and mortality of fry in females were found to be sensitive indicators of toxicity in waste water from a pulp mill. In order to be able to recommend the system proposed, it is also necessary that similar effects can be demonstrated in the field. Fry-carrying females were thus collected from polluted areas and from reference areas.

Table 5. *Occurrence of fry that had died late in Idefjorden and at Stenungsund.*

	Idefjorden -88	*-89*	*Stenungsund -88*			*Stenungsund -89*				
Km, N or S, from the discharge			1 N	3 S	6 S	5 N	1 N	3 S	6 S	12 S
% females with dead fry	24	13	60	50	14	0	8	7	0	0
% dead fry of total number	1.2	5.7	19	26	2.1	0	1.4	1.5	0	0

In October—November 1988 and 1989, viviparous blenny were collected from Idefjorden and Stenungsund (Figure 1). Fishermen in Idefjorden were unable, despite major efforts, to catch viviparous blenny closer to the discharge point at Halden than 7 km. The material was analysed in fresh condition. The occurrence of early-dead fry was higher than in Vendelsöfjorden in 1987 but hardly higher than in the 1988 and 1989 samples from Fjällbacka. However, 24 and 13% of the females had fry that had died in a late stage these years, (Table 5), but no such fry were observed at Fjällbacka or Vendelsöfjorden.

At Stenungsund, test fishing for blenny was done in 1988 and the fry of caught females were checked at three stations in a gradient from the discharge points of the petrochemical industries (Jacobsson and Neuman, 1991). The catches closest to the discharge points were very small, the fish were relatively large and the frequency of late-dead fry was high (Fig. 6). At a distance of three kilometres away, the catches were larger but the frequency of dead fry was still high; it was not until a further three kilometres away that the frequency of dead fry decreased to a significantly lower level, but still not to zero as in the reference areas. The occurrence of early-dead fry did not differ between the stations at Stenungsund and hardly differed from that at Fjällbacka in 1988 and 1989; all three stations were however at a significantly higher level than in Vendelsöfjorden. No differences could be found between the stations at Stenungsund as regards fecundity, i.e., the total number of fry per female in relation to the size of the female.

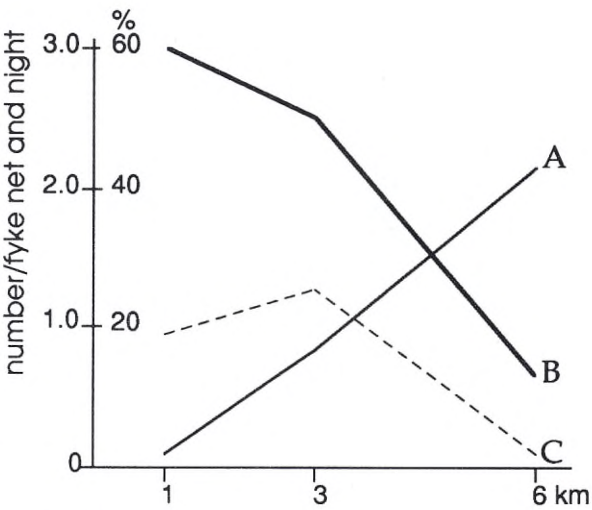

Figure 6. *Abundance and fry mortality at Stenungsund in 1988.*
A — number/fyke net and night
B — frequency (%) of females with late-dead fry
C — frequency (%) of late-dead fry of the total number of fry.

The investigation at Stenungsund was repeated in 1989 over a longer gradient. Recently dead fry were now only found at the two stations closest to the discharge points and the frequency there had decreased strongly (Table 5). The situation had thus improved considerably, which is probably associated with improvements made at the industries concerned.

In 1989, investigations were also conducted in Brofjorden and in the inner parts of the Göteborg archipelago. Females from these areas were investigated in fresh condition but no late-dead fry were found. The occurrence of small fry was compared with the reference area at Fjällbacka (Table 6). Material from Idefjorden and Stenungsund in 1989 were also included. The share of females with small fry diverged significantly ($p<0.05$, chi-2 test) from the levels in Fjällbacka in Idefjorden and at a station near Stenungsund (3S). At those places very few viviparous blenny were present as well as at the station closest to the discharge points (1N) which was probably caused by the high fry mortality in earlier years. Statistical comparisons (chi-2) of the share of small fry of the total number of fry shows that Idefjorden, Göteborg and the two stations closest to Stenungsund (1N and 3S) had clearly higher shares of small fry than Fjällbacka.

In 1989 test fishing was done at Norrsundet 1.0—2.6 km from the discharge point using 24 pairs of fyke nets for one month without one single viviparous blenny being caught. The explanation of this may be a severely disturbed recruitment; recruitment disorders were found in several other species (Neuman and Karås, 1988; Karås et al., 1991).

Table 6. *The occurrence of small fry in the 1989 material.*

		No. of females	% of females with small fry	Total number of fry	% of small fry
Fjällbacka		95	26	4200	0.7
Idefjorden		46	52+	3468	8.4+
Brofjorden		40	25	2992	0.8
Göteborg		150	21	9243	1.4+
Stenungsund	5 N[1)]	32	19	2813	0.3–
- " -	1 N	13	46	1263	2.4+
- " -	3 S	14	57+	1684	3.2+
- " -	6 S	50	36	4846	0.7
- " -	12 S[2)]	50	14	3683	0.2–

[1)] 5 km N of discharge points [2)] 12 km S of discharge points
+ significantly ($p<0.05$, chi-2) larger than at Fjällbacka
– significantly smaller than at Fjällbacka

The mean weights of small and normal fry were compared between areas but it was not possible to demonstrate any clear differences. Thus, in the field studies, the fairly laborious weighing of fry could be eliminated. The results also suggest that neither fecundity nor occurrence of early-dead fry are sensitive measures. On the other hand, the occurrence of small and late-dead fry appears to give good information on pollution effects.

EXPERIMENT INTO TOXIC EFFECTS ON REPRODUCTION

Initially it was mentioned that the suitability of viviparous blenny for emission control programmes had been demonstrated using experiments into the effect of waste-water emitted from a pulp mill on the mortality and growth of fry. The present project has included a similar investigation where effects on gonad development and spawning were studied. In addition, two experiments have demonstrated that captive viviparous blenny spawns and develops fry to what appears to be a normal extent.

Table 7. *Influence of waste-water from a forest industry on the gonad development of females.*

	Concentration of waste-water 0%	0.25%	0.5%	2.5%
Number of female	8	9	8	5
% undeveloped gonads	25	22	25	40

In early June 1988, i.e., more than two months before spawning, viviparous blenny were placed in tanks with continuous supply of different concentrations of waste-water from the pulp mill studied earlier. Details on the experimental technique and the properties of the industry were reported in Jacobsson et al. (1986). The number of females with undeveloped gonads was similar in all concentrations except the highest (Table 7). The low number of fish in this experiment do not, however, permit any significant conclusion to be reached. All fish with developed gonads became fertilized. The experiment indicates that the early stages of reproduction are less sensitive than fry development during pregnancy.

Outside the framework of the project, a reproduction experiment was conducted during July—November 1989 at the Ringhals nuclear power station where effects of chlorinating the cooling water were studied. Chlorinated cooling water was pumped into one tank and unchlorinated into another containing viviparous blenny of both sexes.

The long experimental period enabled the total effect on late gonad development, spawning and embryo and fry development to be studied. Occurrence of early-dead fry was strongly increased in the chlorinated water and all females in such water had small fry (Table 8). However, no fry had died in a late stage. In the chlorinated water, all fry had poor growth; normal fry were only half as large as those in the same category in unchlorinated water.

Table 8. *Chlorination experiment at Ringhals, autumn 1989.*

	No. of females	*Early-dead fry*		*Small fry*		*Mean weight (g)*	
		% females with	*% fry of tot. no.*	*% females with*	*% fry of tot. no.*	*Normal*	*Small*
Unchlorinated water	6	17	0.6	17	1.3	0.39	0.15
Chlorinated water	8	71	7.0	100	26.6	0.18	0.08

CONCENTRATION OF PERSISTENT AND BIOACCUMULATING SUBSTANCES IN FISH

Investigations into contaminants in the marine coastal zone have demonstrated the great difficulty in finding suitable objects for monitoring. On the Swedish west coast it is difficult to find species which both are more or less stationary and at the same time have a size suitable for individual analyses. Normal sampling for analysis of halogenated compounds requires about 10 g of homogeneous tissue such as muscle. As mentioned earlier, flounder has earlier been used in monitoring studies. It does not fulfil the requirement on a stationary way of living, but this problem has been partly avoided by selecting the seasons for sampling. On account of the chemical analyses it is very important that the population studied is representative of the sampling area, i.e., the population remaining in the sampling area. To keep the cost of chemical analyses low it is also important that the analytical matrix represents homogeneous populations, and that the variance in analytical results is small. If this can be fulfilled, then a more reliable estimation of the contamination can be obtained and the number of analyses can be reduced.

Within the Swedish Environmental Monitoring Programme investigations conducted into flounder during the years 1980—1988 at Fjällbacka reveal considerable statistical variation within the annual sample as well as a large inter-year variation, and thus the results lead to uncertainty in the assessments. Table 9 shows mean concentrations of PCB and coefficients of variation. The analyses were made by the Special Analytical Laboratory/ Swedish Environmental Protection Agency. The large variations demonstrate the need to find a more effective analytical matrix for studies conducted at the Swedish west coast.

Table 9. *Total concentration of PCB (extractable fat, mg/kg) in muscle of flounder from the Fjällbacka archipelago.*

Year of collection	*Number*	*Mean value*	*Coefficient of variation*
1980	20	3.6	57
1981	20	1.7	39
1982	20	3.2	71
1983	17	2.0	46
1984	25	1.7	77
1986	25	4.0	36
1987	24	1.5	52
1988	24	2.0	66

In order to study the possibilities for including blenny as a study object in future monitoring activities, we collected blenny during January and July—November, 1988. The intention was to study variation within the population and any seasonal variations. As a comparison with our study area (reference area) we also used fish collected in 1987 in a locally polluted area around Fjällbacka harbour. The latter results have been included in an investigation performed earlier. All the viviparous blenny were analysed at the Centre for Industrial Research (Senter for Industriforskning) in Oslo after preparation at the Swedish Museum of Natural History. The samples collected in 1987 and in January 1988 were analysed for total concentrations of PCB. In order to decrease the expenses for individual analyses and thereby increase their number, the material collected during the period July—November 1988 was analysed for a small number of individual congenes. In this summary, we have chosen to present IUPAC 153 — 2.2´, 4.4´, 5.5´ hexachlorobiphenyl — which is a dominating and apparently stable congene in the PCB spectrum.

Since PCB occurs dissolved in body fat both PCB concentrations as well as the fat content of the analyzed tissue samples have been determined. The concentration of PCB in fish is largely governed by equilibrium partitioning (fat/water) processes. In order to understand the biological processes, it is thus necessary to study the concentration in the organ where the substance is present in dissolved (lipid weight basis) and not on fresh weight basis. As regards fat determinations in material collected in January 1988, there were, however, analytical problems and thus the January material is here presented only as concentrations on a fresh weight basis.

Table 10 shows the PCB concentrations in males and females. The results do not show any difference between the sexes. The table also includes corresponding concentrations in a locally polluted material representing both sexes from the area around Fjällbacka harbour. The material was collected by a local eel fisherman in fyke nets at different places close to the harbour. It can be seen that the concentrations are much higher there than in the study area which is about 5 km from Fjällbacka harbour. The most probable explanation of the local pollution is that the bottom paint often used on small fishing vessels during the 60's and the early 70's contained more than 5 per cent by weight of PCB. Such marked differences measured on a free living fish species in such a restricted coastal area as the Fjällbacka archipelago indicate the potential value of the viviparous blenny as a monitoring matrix in chemical analyses.

Table 10. *Total concentration of PCB (wet weight ng/g), range and coefficient of variation in muscle of viviparous blenny collected in January 1988 in Fjällbacka archipelago. The difference between males and females is not significant.*

Place	*Number*	*PCB*	*Coefficient of variation*
Study area in Fjällbacka archipelago (males)	9	10 (4—21)	51
Study area in Fjällbacka archipelago (females)	9	15 (5—27)	46
Fjällbacka harbour, locally polluted (females+males)	20	150 (24—500)	69

Table 11 and Figures 7 and 8 show the analytical results for IUPAC 153 and the fat percentages in the monthly samples. The results show that there is a very small variation in the concentration of PCB in extractable fat during July—August. The material collected in October shows a strong decrease in fat percentage and at the same time a considerable variation of PCB concentration in the fat. In the November material, the fat percentage has again increased and the PCB levels in the fat are approaching the levels found during July—September. However, there is still a large variation in the November material. The analytical results expressed as values on a fresh weight basis do not vary as much between months. However, the coefficient of variation for the latter values is larger during July—September than the corresponding value expressed on a fat weight basis.

There may be different explanations of the observed situation. Investigations conducted on herring (Bignert et al., 1993) show that concentrations of DDT and PCB show marked increase in fat during reproduction periods when the amount of fat in the fish rapidly decreases and reaches extremely low levels. Variations in fat concentration in non-reproducing periods, however, cause no change in the concentrations of PCB on a fat weight basis. The rapid depletion of fat in the energy reserves during reproduction was suspected by the authors to result in a new equilibrium of partition with ambient water not having time to adjust itself. The same process may also be behind both the considerable increase in concentration and variation in PCB-values on a fat weight basis seen in the present October

Table 11. *The concentration of 2.2´, 4.4´, 5.5´ hexachlorobiphenyl in muscle from female viviparous blenny collected in the study area in Fjällbacka arhipelago during the period July—November, 1988. The range is given within brackets.*

			Conc. in extractable fat (ng/g)		*Conc. in fresh tissue (ng/g)*	
Month	*% fat*	*number*	*Mean value*	*Coeff. of var.*	*Mean value*	*Coeff. of var.*
July	1.85 (1.45—2.95)	10	190 (140—290)	22.6	3.8 (2.1—8.6)	53.2
August	1.45 (1.14—1.76)	8	230 (150—310)	24.0	3.3 (2.0—4.5)	27.8
September	1.53 (1.07—2.25)	10	300 (160—530)	43.7	4.4 (2.5—7.5)	36.8
October	0.44 (0.05—1.32)	10	1900 (300—8400)	133.2	3.5 (1.4—5.7)	38.7
November	1.00 (0.73—1.42)	10	330 (200—890)	60.6	3.1 (2.3—6.5)	43.0

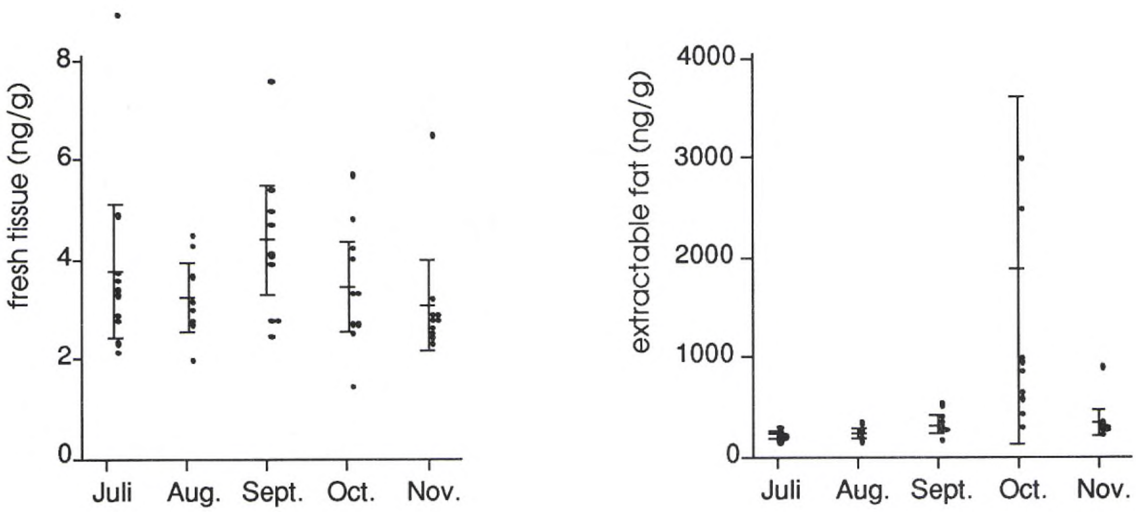

Figure 7. *Concentration of 2.2´, 4.4´, 5.5´ hexachlorobiphenyl in monthly samples of muscle from viviparous blenny.*

material when the fat percentage is very low. That the variation in concentration cannot be read on a fresh weight basis is explained by the dilution phenomenon. If the fat in which PCB is accumulated only amounts to 1.5% of the tissue mass, then weight variations in the fat share will not particularly influence results of measurements based on the entire mass.

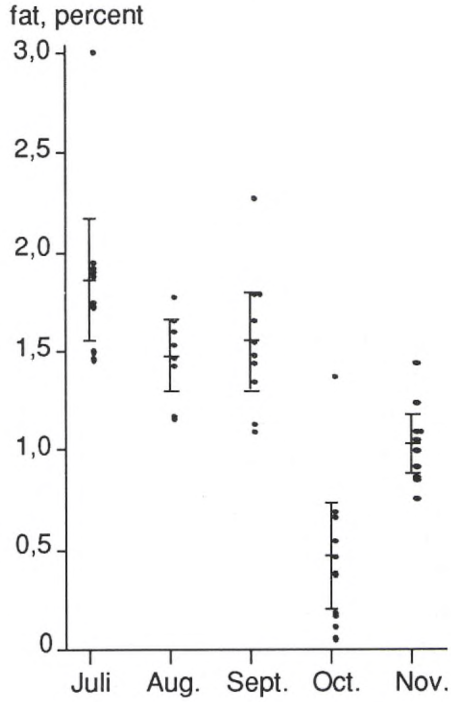

Figure 8. *Fat percentage in the monthly samples of muscle from viviparous blenny.*

The dramatic decrease in fat percentage during October is remarkable and at our present level of knowledge it is difficult to give a reliable interpretation. The situation that the fat value in November is still lower than that during July—September but nonetheless higher than the October value suggests that fry development utilizes particularly large amounts of energy in October; the fry appear to increase most in weight during this period. This situation should be studied further from a biological viewpoint. It is interesting to note that in the study of fry mortality, differences between reference areas and polluted areas took place during a late phase. Seen against the background of the remarkably high concentrations of PCB in fat in viviparous blenny collected during October, this period may tentatively lead to a critical toxic burden on the fry.

The low coefficient of variation for the concentration of PCB in fat during July—August is of interest for monitoring. It is unusual to find coefficients of variation that are lower than 30 in natural fish material. The analysed material was caught at a depth of about 7 m. As discussed in the section on "Seasonal migrations", most blenny stay in deeper water during the summer but return during October and probably also in September to shallow areas. Thus, the species probably does not have such a stationary behaviour during the autumn as during the summer, which would contribute to the wider spread in PCB-concentrations already in september. Another contributing factor is that fry growth starts in September and is at a maximum level in October; reasonably this not only causes low fat concentrations in females but also a large spread in their values (Table 11) and thus also in PCB values.

CONCLUSIONS

The investigations have demonstrated that viviparous blenny is well suited for registering ecologically important effects of toxic substances and for monitoring the occurrence of persistent and bioaccumulating substances. Its unique property among Swedish fish of giving birth to living fry after long pregnancy can clearly be used in environmental monitoring. Sufficient knowledge is now available for elaborating guidelines for biological monitoring on the west coast whereas a programme for the Baltic requires more information on seasonal migrations. A final choice of sex and month for chemical monitoring must be preceded by further investigations of seasonal variations.

The viviparous blenny is a cold-water species which spends most of the year in shallow water (<10 m) on the west coast. During the summer, most fish migrate down to slightly deeper water (<20 m), but return during the autumn to places they lived in before the summer. Despite comprehensive tagging, it was not possible to record any long migration. Thus, the species must be considered to fulfil reasonable demands on stationary behaviour. Along the Baltic coast, the vertical dispersal is larger during the autumn and migrations are insufficiently known.

Abundance variations appear to be easy to follow with simple inexpensive test fishing methods during October, which is a suitable time for study of fry survival. Relatively small variations in catch in time and space allow a moderate input.

Reproduction biology does not appear to differ between the Baltic and the west coast and neither between northern and southern sites on the west coast.

Studies in polluted areas, as well as experiments with industrial wastewater, have demonstrated that occurrence of fry with growth disorders and mortality during a relatively late phase of pregnancy are good indicators of effect, whereas fecundity and death in early stages of pregnancy are less sensitive in the types of receiving waters studied by us. Recruitment disorders have been linked to very low abundances of adult fish which demonstrate long-term influence and indicate stationary behaviour.

In order to illustrate the suitability of viviparous blenny as a study object in chemical analysis of contaminants, females were collected monthly during July—November. The occurrence of the non-polar PCB group of substances was determined and the concentrations in fish fat indicate a remarkably low variation during July—August. During this period, the coefficient of variation is clearly lower than what is normally registered in fish. Later during the autumn — after spawning — the variation increases. Particularly during October, it is large. This coincides with a very strong reduction in the amount of fat in fish which is probably an effect of the female´s energy requirement during a period when fry growth is largest.

Continued investigations should be conducted mainly within three sectors:

- 1. The final choice of sex and month for the contaminant monitoring programme requires verified analysis of fat concentrations during an annual cycle with complementary analysis of PCB.
- 2. Increased understanding of reproduction strategy and fry mortality in polluted areas would be obtained if the fat analysis according to 1) were combined with control of fry growth and fat concentration. Degradation of fat reserves in adults during energy-requiring periods leads to a release of harmful substances and possibly a critical exposure for the fry in the female.
- 3. Seasonal migrations in the Baltic should be studied with test fishing and tagging.

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