Considerable evidence has accumulated in the last decade to show that sex determination in several fish species can be affected during larval development by environmental factors such as temperature \(^1\)–\(^5\) and pH \(^6\),\(^7\).

Marbled sole \(\textit{Limanda yokohamae}\) has a known XX-XY sex determination system, and gynogenetic fish develop as phenotypic females.\(^8\) Development of gynogenetic fish to phenotypic females typically occurs when fish are held under natural temperature conditions.\(^9\) However, some Pleuronectiformes, such as the hirame flounder \(\textit{Paralichthys olivaceus}\) and the barfin flounder \(\textit{Verasper moseri}\),\(^1\)\(^0\) have temperature-dependent sex determination, suggesting that other members in this order may also employ this strategy. Such a sex determining system in fish is of obvious benefit for aquaculture. Indeed, hormonal treatments can be used to control phenotypic sex in many species,\(^1\)\(^2\) but these treatments may pose hazards for health or environment.\(^1\)\(^3\) The establishment of ‘clean methods’ of sex control is therefore desirable for cultivated fish species. Environmental factors, such as temperature, are possible examples of these methods. The purpose of this study was to test this hypothesis, by evaluating sex ratios after manipulation of the temperature during the larval stage of marbled sole.

Fertilized eggs of marbled sole were acquired from a few breeding males and females held together in a 1 m\(^3\) tank at the Hokkaido Institute of Mariculture, Shikabe (spring). Fish were reared at ambient seawater temperatures in flow-through seawater tanks, with larvae being fed \(\textit{Brachionus}\) and \(\textit{Artemia}\) nauplii, while juveniles were fed artificial diets. When ambient seawater temperature reached 15°C (summer), the experimental water temperatures were maintained at this level (15 ± 2°C) or 25°C. Three groups of 150 fry from the same stock were placed in separate tanks and fed for different rearing periods at low temperature as follows (Fig. 1). In the first group, the temperature was 15 ± 2°C from the start to the end of experimentation (group A). The second group was reared at 15 ± 2°C until 115 days after fertilization (mean total length (TL) of 25 mm), whereafter the temperature was gradually shifted to 25°C over 10 days (group B). The third group (group C) was reared at 15 ± 2°C for 146 days (mean TL of 35 mm), and then transferred to 25°C, as for group B. Just before and at the end of the experiment, a sample of 10 fry was removed, and body lengths were recorded. On day 304, all fish were removed and preserved in Bouin’s solution for sexing.

At the time of sexing, intersexual gonads could not be observed histologically in any of the groups. The ovaries contained a clear ovarian cavity and oocytes were in the peri-nucleous stage, while the testes had a mesh structure corresponding to presumptive seminal lobules. The sex ratio in group A was 52.9% females while groups of fish shifted from 15 ± 2°C to 25°C at a mean TL of 25.2 ± 3.42 mm (group B) or 35.6 ± 3.89 mm (group C) produced 17.9 and 42.9% females, respectively (Table 1). The difference in sex ratio between groups A and B was statistically significant (\(P<0.05\)). Survival ratios in groups A, B and C were 75.3, 64.3 and 68.0%, and hence, were not affected by temperature regimes (\(P<0.05\)).

Our results demonstrate that the sex ratio inclined towards male if larvae of 25.2 mm in mean TL are gradually acclimated to a temperature of 25°C. Previous reports on marbled sole indicated that, between 14°C and 24°C, gonadal sex differentiation became structurally noticeable in larvae from 20.7 to 24.8 mm in TL.\(^1\)\(^4\) Ovaries in larvae of this stage were characterized by the
Sex determination in *Limanda yokohamae*  

It appears, then, that the male sex (XY) is more stable under high temperature conditions than the genetic female sex (XX) which readily manifests itself as a phenotypic male, so that temperature-dependent sex determination in marbled sole held at high temperature regimes resembles that of other flounders.

Although induction of biased sex ratios by water temperature has been recognized in many species, the mechanisms affecting sex determination are still unknown. Under natural conditions, the phenotypic sex is determined by genetic factors, while under extreme conditions, the phenotypic sex is determined by a summation of genetic and environmental factors. Among the Pleuronectiformes, this hypothesis cannot be applied to genetic males, but genetic females only, since there are no reports of female-biased sex ratios.

We thank Dr Mark Lokman, University of Otago, New Zealand, and Mr M. R. Nader, University of Hokkaido, Japan, for their critical reading of the manuscript. This study was supported in part by a Grant for Scientific Research from the Fisheries Agency, Japan and Hokkaido, and by a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

**REFERENCES**


