REPRODUCTIVE BIOLOGY OF GIANT KOKOPU, *GALAXIAS ARGENTEUS.*

Wylie M. J., Closs G. P., Lokman P. M.

Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand. Fax +64-34797584, e-mail: wylma731@student.otago.ac.nz

Introduction:

In New Zealand, the whitebait fishery is comprised of five amphidromous species of crystalline juveniles that belong to the genus *Galaxias*, a group of Southern hemisphere Salmoniformes that have a marine larval feeding phase. Whitebait is a seasonal and valuable delicacy [6,7]. Progress towards the commercial production of whitebait using *G. maculatus*, the most abundant galaxiid species [7,9], has been slow due to technical constraints (e.g. low fecundity and poor larval survival). The giant kokopu *(G. argenteus)*, is a promising alternative, although it is considered threatened [8]. Specimens over 40cm in length and 1 kg in weight have been recorded [2], with a far greater fecundity and egg diameter compared to *G. maculatus* [8,11]. However, no observations of spawning or egg deposition have been documented [5] and essentially nothing is known about the reproductive physiology of this fish. To fill these voids, oocyte diameters and plasma levels of estradiol-17β (E_2) were determined from wild fish at monthly intervals throughout the reproductive cycle until ovulation. Ovarian mRNA levels of cathepsin D, a protein associated with yolk processing in developing oocytes [3,4], were also measured. In order to assess the potential of this fish for cultivation, the same data were obtained from fish held in captivity via repeated biopsies. The outcomes were compared, and where possible, gametes collected from wild and captive fish to obtain data on fertilization and hatching rates following incubation of eggs at a range of different salinities.

Methods:

Wild fish - Ovarian tissue was biopsied and blood samples were collected from four to six wild fish (~300 g) at monthly intervals until ovulation. *Captive fish* - The first group of wild-sampled fish were transferred to a holding facility and maintained under simulated natural photoperiod and water temperatures. Ovarian tissue was repeatedly biopsied and blood samples were collected at near-monthly intervals until ovulation. Subsequent to sample collection, oocyte diameters were measured using light microscopy. Methacrylate resin sections were stained with haematoxylin and eosin to determine the developmental stage of the oocytes. Cathepsin D mRNA levels were quantified using real-time PCR and plasma E_2 levels were measured using radioimmunoassay. *Artificial propagation* - Mature fish were checked for ovulation daily. Stripped eggs were dry-fertilised with milt from wild fish prior to activation of gametes in water of different salinities (0 ppt, 9 ppt, 15 ppt and 30 ppt). Water was subsequently drained from treatments and eggs were incubated semi-dry at 10 ± 1°C under 100% humidity. In an additional treatment, eggs were fertilised and incubated fully submerged in local dechlorinated tap water. Dead eggs were removed daily. Hatch rates were calculated for each treatment.

Results and Discussion:

Oocyte growth started in December and continued in wild fish until late June, when ovulated eggs could be obtained. Levels of E_2 increased as vitellogenesis progressed, followed by a dramatic reduction to undetectable levels on completion of oocyte growth, a pattern that is reminiscent of that in salmonids. Messenger RNA levels for cathepsin D increased as vitellogenesis progressed in giant kokopu. In contrast, mRNA levels were highest at the onset of vitellogenesis in gilthead sea bream *(Sparus aurata)* and rainbow trout *(Oncorhynchus mykiss)* [3,4]. In captivity, fish also reached the ovulatory stage, but it lagged wild fish by more than six weeks, presumably reflecting the stresses of repeated biopsy and a protozoan infection in March; indeed, stress through the action of corticosteroids can reduce immunocompetence and alter levels of hormones [1]. High levels of cathepsin D seen during mid vitellogenesis may similarly reflect fish illness, as over-activity has been associated with poor-quality eggs [12]. Viable eggs were collected from both groups and indications of compromised egg quality in captive fish were further reinforced by low hatch rates (26%), especially when compared to hatch rates of submerged eggs from wild fish (71%). Regardless of salinity, incubation of eggs under semi-dry conditions was unsuccessful due to an unidentified disease.

Conclusion:

Oocyte growth and estradiol-17β plasma levels were documented throughout the reproductive cycle of giant kokopu until spontaneous ovulation in both wild and captive fish. We conclude that captive fish ovulated and produced viable eggs, but later in the season than fish from the wild. Larvae only hatched from eggs fertilised and fully submerged in fresh water until hatching, and hatch rate was higher in eggs from wild fish than from captive fish. In addition to the greater fecundity and larger egg size of *G. argenteus* compared to *G.*

maculatus these novel results provide a foundation for which the controlled propagation of the species can be optimised for both conservation and aquaculture purposes.

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