Biology of the Black-stripe minnow *Galaxiella nigrostriata*, (Galaxiidae) in an acidic, black-water lake in Melaleuca Park near Perth, Western Australia

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Abstract – Individuals of *Galaxiella nigrostriata* (Shipway) from acidic, temporary, black-water wetland designated EPP 173 at Melaleuca Park, Ellen Brook, Western Australia, are compared with members from the main area of distribution of the species some 350+ km further south in Western Australia. They were found to be similar to the southern populations in size frequency distribution and habitat. Adult fish from EPP 173 were first sampled, and breed, one month later than those from southern sites. Diets of all populations were similar, comprising predominantly microcrustaceans, dipterans and rotifers; however, prey items at the water surface were consumed less commonly by the northern specimens. *G. nigrostriata* was found to be sensitive to high water temperatures. Laboratory trials showed that the fish cannot tolerate, for more than a few seconds, temperatures >26°C but preferred a temperature of 14.5°C. The survival of the *G. nigrostriata* population in EPP 173 is related to the strong thermal stratification which develops on hot days, with the surface water temperature typically reaching values >26°C but overlying cooler bottom water around 15°C which provides a cool water refuge for the resident fish population. The northern outlier population in EPP 173 occurs in the path of the urban expansion of Perth and, since it has high intrinsic conservation value, its management for conservation is paramount.

Keywords: Black-water, limnology, thermal stratification, Galaxiidae, feeding

INTRODUCTION

Galaxiella nigrostriata (Shipway, 1953) (Teleostii: Salmoniformes: Galaxiidae) is a small (maximum length ~4 cm) teleost from acidic, black-water temporary pools in the extreme southwestern corner of Australia (Figure 1). *Galaxiella nigrostriata, Galaxiella munda, Lepidogalaxias salamandroides* (Lepidogalaxiidae), and *Nannatherina balstoni* [Nannopercidae, following the current Family classification of Allen *et al.*, (2002)] are four species of freshwater teleost endemic to southwestern Australia. They have attracted scientific attention because of i) their specialised habitat within a very restricted geographic distribution, and ii) their method of surviving the seasonal drying of their habitats by aestivating in moist subsurface substrates (Christensen 1982; McDowall and Pusey 1983; Berra and Allen 1989, 1991, 1995; Leung 1988; Berra *et al.* 1989, 1990; Pusey 1989, 1990; Pusey and Stewart 1989; Pusey and Edward 1990; Jaensch 1992; Pen *et al.* 1993; Martin *et al.* 1993; Gill and Neira 1994, Morgan *et al.* 1996, 1998). Most populations of all four species occur within a narrow strip of peat flats between Augusta and Albany, southwestern Australia, but three species have at least one outlier population (by some 350 km) north of Perth (Figure 1). Knowledge of these outlier populations is based on recent records: *G. nigrostriata* near Bunbury (Morgan *et al.* 1996, 1998) and Melaleuca Park (Knott and Jasinska 1996); *Galaxiella munda* (McDowall) (Galaxiidae) between Gingin and Muchea (Allen 1982); and *Nannatherina bolstoni* (Shipway) (Khulidae) at Gingin (Morgan *et al.* 1996, 1998). The similarity of their disjunct distributions indicates that all three species may once have been more widespread in southwestern Australia.

The northern outlier population of *G. nigrostriata* occurs in an un-named wetland, designated as EPP 173 (Environmental Protection Policy Map Identification Number) (Hill *et al.* 1996)] within Melaleuca Park, some 25 km northeast of Perth,
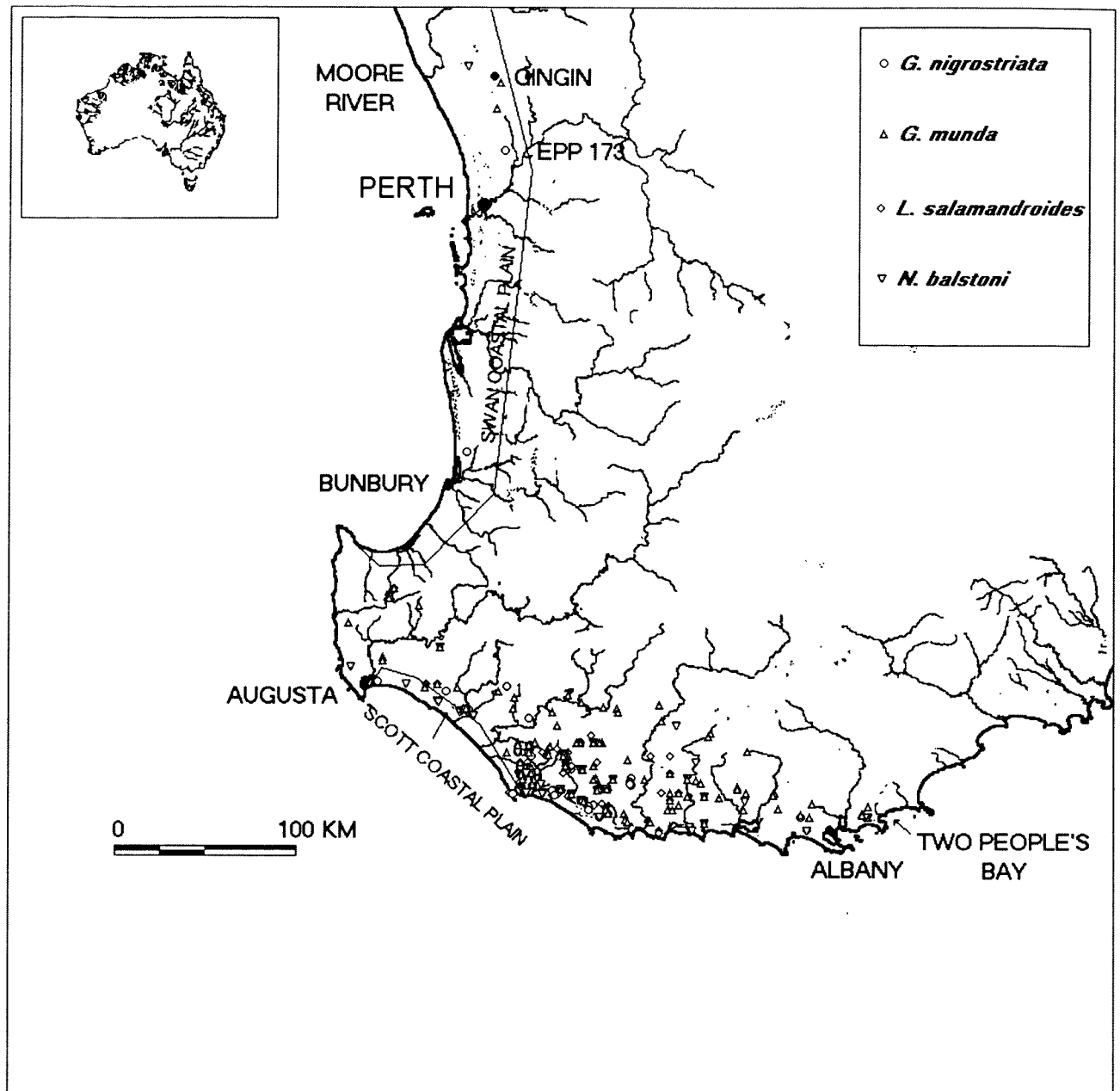
Figure 1   Distribution of *Galaxiella nigrostriata*, *Galaxiella munda*, *Lepidogalaxias salamadroides* and *Nannatherina bolstoni* within southwestern Australia.

Western Australia. Given the significantly higher day-time temperatures, particularly in summer, and lower annual rainfall at EPP 173 (31°C and 690 mm p.a., respectively) compared with the area between Augusta and Albany where *G. nigrostriata* mainly occurs (25°C and 1200 mm p.a., respectively) (meteorological data from the Bureau of Meteorology 1999), aestivation, emergence time, size structure of the population, behavioural biology and diet of *G. nigrostriata* at EPP 173, were investigated. The results are compared with published data from the southern populations (Pen *et al.* 1993; Morgan *et al.* 1996, 1998). Experimental evidence is presented supporting the hypothesis that this northern outlier population has survived

due to the specific hydrology of EPP 173. From these findings, an attempt is made to elucidate some of the factors which might limit the distribution of *G. nigrostriata*. This paper is part of a series of three concerning the outlier population of *G. nigrostriata*. In the other papers in the series, Knott *et al.* (2002) describe the unique limnological conditions in EPP 173 and Smith *et al.* (2002) describe the molecular and morphological relatedness between the outlier and southern populations.

This outlier population of *G. nigrostriata* has a strong claim for conservation, but longevity of the population is threatened from three sources: urban expansion, groundwater extraction and highway

construction. The paper concludes with an appraisal of these threats and the series contributes to knowledge of the southwest Australian piscifauna.

Study Area

The environment of EPP 173 (31°42'18"S; 115°57'41"E; elevation 50.41 m AHD (Australian Height Datum); maximum depth 70 cm; ~1.5 ha in area) is described in detail in Knott *et al.* (2002) but features salient to this paper are briefly summarised here. There is no surface drainage into EPP 173; the only other surface features are a small outflow stream to the east, and a smaller, temporary water body (Mb), contiguous with its northern margin. EPP 173 is fed *via* a permanent spring seepage which maintains an area of several square metres of boggy peat and shallow (<11 cm deep) surface water in late summer and autumn when nearby wetlands are completely dry. EPP 173 and Mb are continuous at times of peak winter levels of infilling. At EPP 173, water: salinity is low (444 mS cm⁻¹), pH decreases from 5.5 during inundation to 3.25 – 3.8 during the drying phase and colour is very dark ($\geq$ 1200 TCU). Water temperatures reflect the input of cool (~15°C) ground-water, and in summer, solar heating. Surface water temperature range from ~27°C in summer to 13°C in winter (Knott *et al.* 2002). In winter, the water column is homogenous with respect to temperature but in late spring/early summer, strong thermal gradients develop, for example: 26.7°C within 2 cm of the surface to 15°C at 65 cm depth (15 November, 1995; Knott *et al.* 2002).

MATERIALS AND METHODS

Aestivation and emergence

On May 12, 1996, while the swamp was still dry, a trench (200 cm x 40 cm x 60 cm deep) straddling 2 microhabitat-types [a stand of *Baumea articulata* (Cyperaceae) and a winter open water habitat] was dug in the peat near the central area of EPP 173. The trench was filled with rainwater and the soil removed was sieved (2 mm² mesh size) to check for aestivating *G. nigrostriata*. The water-filled trench was swept with a fish net for four hours (11.00–15.00 hours) for emergent fish. Root mats in 30 cm³ blocks of soil were removed and submerged in aquaria to observe any fish emergence from that microhabitat. In late May, 1996, leaf litter, the underside of logs, as well as four blocks of sandy/peat substrate (40 cm x 40 cm x 60 cm deep) from EPP 173 were searched for aestivating fish by sieving the detritus. To record where fish aestivated and to exclude the possibility that the fish re-colonised EPP 173 each year from nearby refuges, for example by swimming upstream through the

outflow channel, EPP 173 was isolated from the small auxiliary swamp, Mb, by a 30 000 x 40 cm fence of 1 mm² mesh, supported by star-pickets and inserted 10 cm into the substrate, and from the outflow stream 6 m from EPP 173 by a fence of the same mesh.

Size structure

Low numbers of animals were collected from EPP 173 to minimise the impact on the small *G. nigrostriata* population. The same individuals were used in as many aspects of the study as possible. A sample of *G. nigrostriata* large enough to construct size frequency histograms was collected on August 19 and September 19, 1996. Fish were anaesthetised in 0.015 % benzocaine solution. Total length (snout to end of tail fin) was measured using dial vernier callipers (0.1 mm increment). Only recently killed or anaesthetised fish were measured to prevent errors due to deterioration of the specimens.

Diet

Sample sizes for diet analyses are low because preserving specimens for this purpose precluded their use in other components of the study. Stomach contents were analysed as follows: numbers (+ sampling date), *ex* EPP 173: 3 adults (26 October, 1995); 12 larvae (6.7–13.3 mm) (19 August, 1996); 12 larvae and juveniles (7.08–20.72 mm) (1 October 1996); *ex* pools along Chesapeake Road, southeast of Northcliffe (34°42'S, 116°07'E): 2 adults (July, 1996); 6 (adults) (9 September, 1996). The stomach contents were identified using dissecting and compound microscopes. The diets were analysed by frequency of occurrence, describing the relative contributions of each kind of prey to the total number of prey-items within each stomach.

Laboratory experiments

Two experiments were conducted to compare the behaviour of *G. nigrostriata* from EPP 173 in aquaria with and without an established thermal gradient. Two un-aerated aquaria measuring 60 x 25.5 x 30.5 cm (length x width x depth), were filled to a depth of 28 cm with water from EPP 173 (collected in August 1996) and placed in a controlled temperature room at 17.5°C. Two treatments were established, a control and an experimental. In the control treatment, the water column was only slightly stratified with respect to temperature, 16.5°C at the bottom, varying between 17.0 and 19.5°C at the surface due to the heating effect from the light source. In the experimental treatment, a more pronounced temperature gradient was established by placing an aquarium heater (Tronic 200 Watt) horizontally and 1 cm below the water surface, whilst standing the aquarium on brick supports within an ice bath; the ice was maintained level with the bottom of the aquarium. In both, a

100 Watt light source was suspended 15 cm above the water surface to maintain constant illumination levels between treatments. Water temperature was measured using a 0–50°C E-Mill Precision thermometer (0.1°C graduated increments). In the experimental treatment, water temperatures 2 cm below the surface ranged between 24.5 and 28.0°C; water temperatures 2 cm above the bottom ranged between 8.0 and 9.5°C.

In the first experiment, fish were released individually at the surface from a net into the stratified aquarium to acclimatise for 1 hour. The position of each fish relative to the bottom of the aquarium was then recorded at three random times during the second hour. The same fish was then transferred to the control aquarium and the same procedure repeated. Eleven replicates were performed over 2 days, using a different fish in each trial. The three scores from each trial were averaged to give a mean position within the temperature gradient for each fish in each treatment. The data were analysed using a paired two-tailed t-test with 11 degrees of freedom. The second experiment compared the amount of time fish spent feeding at the surface in each temperature treatment. Collembola, which are a natural component of the diet of this species, were collected from a suburban wetland near Perth. Twelve trials were conducted using 12 different fish and the sequence of treatments to which each fish was subjected was alternated in each trial. After fish had acclimated for one hour, a 2 cm² aggregation of Collembola estimated to consist of approximately 400 animals, was added to the aquarium surface. For 15 minutes the amount of time each fish spent within 3 cm of the water surface was recorded.

RESULTS

Aestivation and emergence

In 1996, no fish were recovered in EPP 173, regardless of the search method, prior to inundation following the onset of winter rains. The first specimens in 1996, 4 adults (length, 25.5 – 34.0 mm), were recorded on June 29 when the water depth in the lake at the site of permanent seepage was 64 cm. On August 19, 1996, 17 G. nigrostriata 0+ recruits (length, 6.7 – 13.3 mm) and on September 19, 1996, 35 G. nigrostriata 0+ recruits (length, 7.0 – 20.7 mm) were collected from EPP 173 and also from Mb, when it was still separate from EPP 173. No G. nigrostriata were caught in the outflow stream downstream from the fence.

Size frequency

Seventeen specimens from EPP 173 (length, 6.7 – 13.3 mm) collected 19 August, 1996, and 35
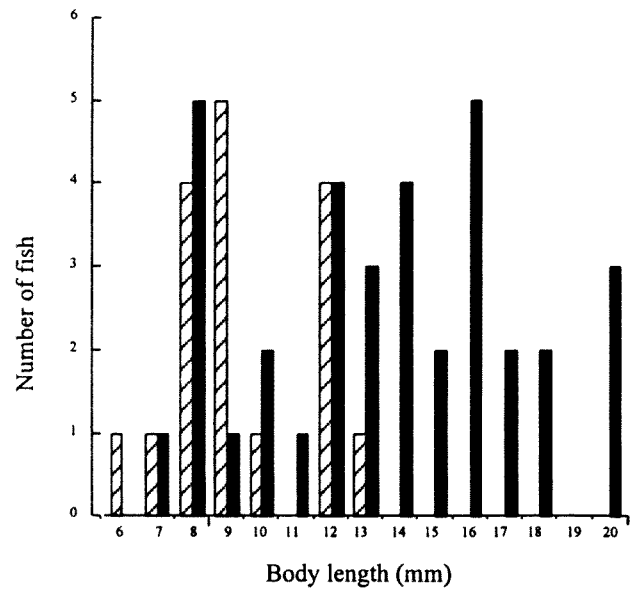

Figure 2 Length-frequency histogram of two samples of *G. nigrostriata* from EPP 173 collected in 1996. August, hatched bars; September, solid bars.

specimens (length, 7.0 – 20.7 mm) collected 19 September, 1996, were all 0+ recruits (Figure 2).

Diet

A total of 11 prey taxa were recorded, eight from the stomachs of specimens from EPP 173 (Figure 3) and seven from the Chesapeake Road specimens (Figure 4). Comparisons of the stomach contents
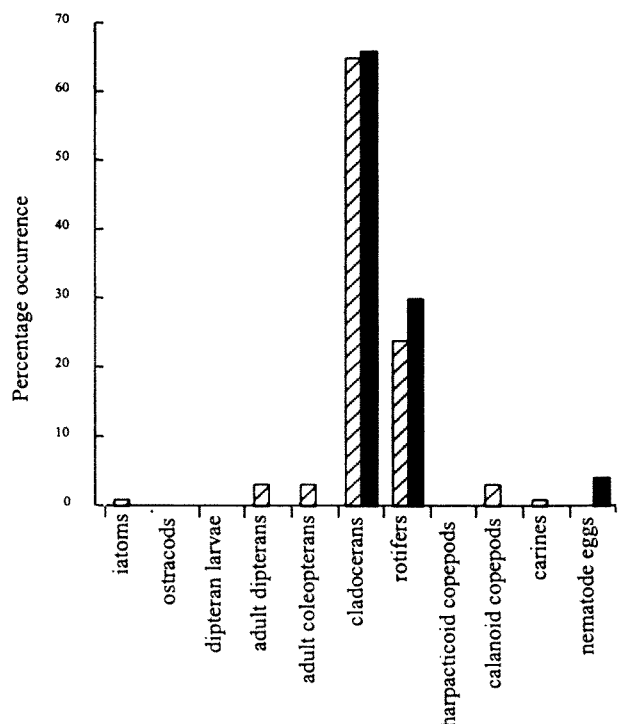

Figure 3 Combined percentage occurrence of prey taxa in stomachs of *G. nigrostriata* from EPP 173. Hatched, October 1995. Solid, August 1996.
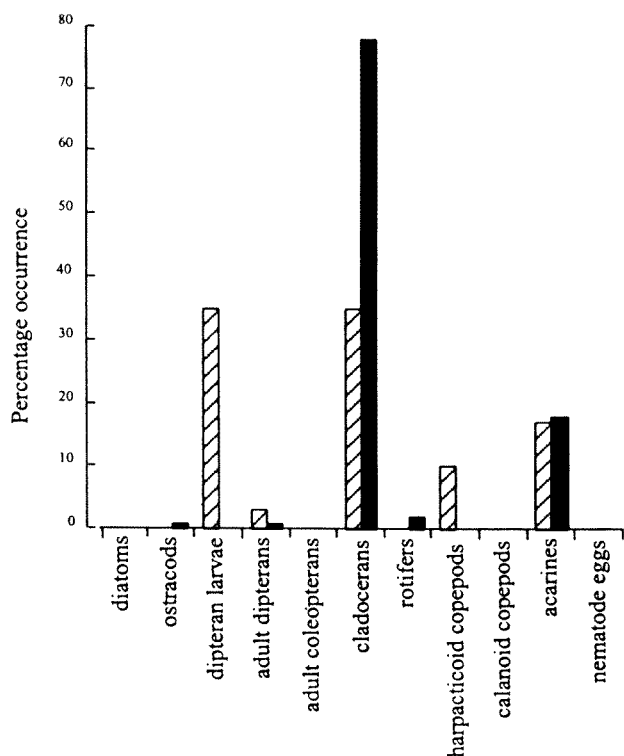
Figure 4   Combined percentage occurrence of prey taxa in *G. nigrostriata* from Chesapeake Road in the lower southwest during 1996. Hatched, July. Solid, September.

from both sites reveal no marked differences in abundance and diversity of prey consumed. Cladocerans, followed by rotifers (EPP 173) and terrestrial insects (Chesapeake Road), were the prey most commonly ingested at both sites. Harpacticoid copepods and acarines also occurred consistently in the diet of the southern fish. Other prey items, such as diatoms and calanoid copepods were recorded infrequently.

Laboratory experiments

Fish were consistently observed at greater depths in the stratified, than in the control, aquaria (Table 1).

Table 1   Average distance (cm) of *G. nigrostriata* from the bottom of the water column in stratified and unstratified (control) aquaria.

Trial	Stratified	Unstratified
1	3.7	17.5
2	3.7	8.7
3	5.7	12.3
4	4.3	7.5
5	4.3	16.7
6	4.3	7.7
7	4.5	6.7
8	2.7	16.0
9	2.5	4.8
10	2.3	14.0
11	5.7	2.7
Mean $\pm$ S.E.	4.0 $\pm$ 1.2	10.4 $\pm$ 5.1

Table 2   Time (seconds) spent feeding within 3 cm of the water surface in the stratified (S) and unstratified (C) aquaria.

Trial	First treatment	Stratified	Control
1	S	1	150
2	C	0	516
3	S	0	170
4	S	3	201
5	C	2	185
6	S.	0	271
7	S	0	0
8	C	0	63
9	S	7	28
10	C	1	0
11	S	1	9
12	C	17	205
Mean $\pm$ S. E.		3 $\pm$ 5	150 $\pm$ 149

The mean position of fish in the stratified aquarium was 4.0 cm ($\pm$ 1.2) from the bottom of the water column, where water temperature was 14.5°C. The average position of fish in the control aquarium, where thermal stratification was not established, was 10.4 cm ($\pm$ 5.1) from the bottom at a water temperature of 17.0–18.0°C. The two results were significantly different (p = 0.003, d.f. = 10; paired two-tailed *t*-test with 11 degrees of freedom).

Similarly, fish in the control aquarium spent significantly more time feeding at the surface than did those in the stratified aquarium (Table 2). Most fish in the control aquarium frequently remained within the top 3 cm of the water column (average time 2.5 minutes), feeding constantly on prey floating at the water surface throughout the 15 minute trial period and making no attempt, subsequently, to increase their depth. In marked contrast, the fish in the stratified aquarium spent most of the time near the bottom of the water column, darting to the water surface to capture prey and then quickly returning to the bottom. The average time each fish spent within 3 cm of the water surface was less than 3 seconds, with the exception of one trial where the fish remained in the epilimnion for up to 7 seconds.

DISCUSSION

Although many wetlands of the Swan Coastal Plain have been degraded (Wrigly *et al.* 1988; Halse 1989) or lost (estimated as 70%, Halse 1989) under the impact of the European presence in Western Australia, numerous wetlands remain and 40 in the vicinity of Perth have been the subject of a major study (summarised in the seven volume series *Wetlands of the Swan Coastal Plain* edited by S. A. Balla and J. E. Davis, released during the years 1993 – 1996). The occurrence of outlier populations of three species of endemic fish known previously

only from the extreme southern area of Western Australia probably indicates that all three once had wider geographic distributions in the southwestern corner of the State. The comparatively recent discoveries of these outlier populations raise the question of how many more outlier populations may occur, even in close proximity to Perth. It is relevant, therefore, to question what limnological features may have facilitated the survival of *G. nigrostriata* within EPP 173. The main habitat of the Black-stripe minnow is temporary swamps along the coastal strip between Albany and Augusta, with the centre of distribution the peat flats along Chesapeake Road, D'Entrecasteaux National Park (Allen 1982; Morgan *et al.* 1996; Pen *et al.* 1993). The northern outlier population in EPP 173 also inhabits an acidic, black-water temporary swamp, but under markedly more stressful (lower rainfall: 690 mm p.a. compared to 1200 mm p.a.; higher summer maximum temperature averages: 31°C compared to 25°C) climatic conditions.

The presence of surface water throughout summer in EPP 173 is not a critical factor for the persistence of *G. nigrostriata* at this site, since i) no fish have been found in the permanent pool when the major part of the wetland has been dry, and ii) fish were present in Mb when it had been isolated from EPP 173 by a mesh fence. Nor is the outflow stream involved in the annual recolonisation of EPP 173 by *G. nigrostriata*. The Black-striped minnows within EPP 173 and Mb presumably aestivate within the boundaries of the swamp beds and therefore comprise a locally resident population.

Clearly acid, temporary waters are important for defining the ecophysiological niche of *G. nigrostriata*. The results presented here indicate two other features essential for the survival of this galaxiid in EPP 173: one, the input of cool (14°C) ground water; two, the high colour of the water, through which a strong temperature gradient is established during the hot days at the base of which is a refuge of cool water (Knott *et al.* 2002). Temperature stratification has also been documented for Lake Jandebup, <10 km to the west of EPP 173, but in that lake the temperature gradient does not exceed 6.6°C (Ryder and Horwitz 1995), possibly due to the water being only slightly coloured, with values between 2 to 17g_{440} m^{-1} (Sommer and Horwitz, 1999). The extremely dark (>1200 TCU) water of EPP 173 facilitates the development of a strong thermal stratification on hot days. When Black-striped minnows are netted and lifted through the warm epilimnion, they die instantly. Consistent with this is the observation that on hot days no specimens of *G. nigrostriata* have been observed near the water surface. This stratification, with the refuge of comparatively cool bottom water is undoubtedly one of the important factors which

has enabled *G. nigrostriata* to persist in EPP 173 despite the high air temperatures.

The heat stress experiment shows that *G. nigrostriata* from EPP 173, when provided with a cool water refuge, avoid water temperatures >26°C which effectively excludes them from the surface layers. However, *G. nigrostriata* in D'Entrecasteaux National Park have been recorded in drying pools at a temperature of 34°C (Morgan *et al.* 1996, 1998). It is unclear from the available data whether there are different responses to temperature shown by the minnows from the two areas, however one might suspect *a priori* that gravid females or juveniles may be particularly susceptible to thermal stress. Thermal avoidance may also reflect scope for exercising choice: *Galaxias maculatus* avoids a plume of warm water discharged from a power station (Boubee *et al.* 1991). The predation rate on flying ants and adult Diptera, i.e. on terrestrial insects, varied seasonally in the southern populations [from 50% (in winter) to 20% (in summer) (Pen *et al.* 1993)] but there was limited predation on terrestrial insects at EPP 173 (<5% of total stomach contents). Results from the heat stress and feeding experiments indicated that the minnows can change their feeding behaviour in response to adverse temperatures at the water surface.

The effects of the warmer, dryer climate experienced at EPP 173 compared with the climate throughout the main zone of distribution of the species are evident in delays in the time of emergence of the larvae and of spawning. On July 8 1991, the predominant size class of 0+ recruits was 5 – 10 mm in the lower southwest (Pen *et al.*, 1993). In 1996, this did not become the modal size class at EPP 173 until late August. Furthermore, in September 1991, 0+ recruits ranged up to 35 mm in length in the lower southwest, but during this month in 1996 at EPP 173, no specimens larger than 20 mm were recorded. Spawning commenced in June in the southern populations in 1991 but in 1996 probably did not commence until July at EPP 173, since gravid adults were not captured there until late June. Although different years have been compared, and conclusions have been based on small sample sizes, we have not detected any evidence for biological divergence between the EPP 173 and the more southern *G. nigrostriata* populations other than the delayed emergence and subsequent spawning by adults .

We think it unlikely that EPP 173 was the only wetland of the central Swan Coastal Plain to have harboured *G. nigrostriata* until comparatively recent historical times, since dark water swamps are believed to have been well represented in wetlands of the Swan Coastal Plain before the arrival of Europeans. Now, however, these swamps occur primarily along the lower south coast. Most of the Swan Coastal Plain wetlands subjected to high

levels of disturbance are now colourless, open water systems from which *G. nigrostriata* may have been excluded by the loss of cool, bottom water refuges stemming from reduction in both colour and thermal stratification. Clearly, the population of *G. nigrostriata* at EPP 173 has high conservation status and stringent management protocols need to be established to conserve the population. Management of the groundwater supply and also of the vegetation and buffer zones surrounding the wetland will be essential.

EPP 173 is threatened from three sources. 1). The urban population in the immediate vicinity is expected to increase from 8 200 residents (1993) to 95 000 (2021+) (Department of Planning and Urban Development 1994). 2). Wetlands within Melaleuca Park are supported by seepage from the Gnangara Mound, an extensive unconfined aquifer which currently provides nearly 40% of the public water supply for Perth, a figure likely to increase to >50% over the next 15 years (Department of Conservation and Land Management 1999). Increasing groundwater abstraction has resulted in the loss of wetlands on the Swan Coastal Plain by the lowering of the water table and hydraulic heads of springs and seepages and although EPP 173 is identified as an ecologically significant wetland to be managed to an Environmental Water Requirement of 0.1 m above ground level (Water and Rivers 1997), experience suggests that it will be difficult to sustain this objective against climatic vagaries and the demand over time for water. 3). Of four alignments considered for the proposed Perth – Darwin National Highway, that most recently preferred is for construction of a highway <3 km east of EPP 173 (Department of Planning and Urban Development 1994); at present, the wetland, 100 m from the edge of Melaleuca Park gains some protection from frequent human visitation by the low intensity rural buffer further the east, with the nearest house some 440 m distant. The site will require management to preserve conditions for the water supply to be maintained, and for the water to continue stratifying on warm days, including maintenance of the vegetation buffer against wind mixing (Knott *et al.* 2002).

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