Short communication

Reproductive ecology and diet of a persistent *Ameiurus melas* (Rafinesque, 1820) population in the UK

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Introduction

The black bullhead *Ameiurus melas* was introduced to Europe from North America in the early 20th Century and invasive populations are now present in many European countries (Novomeská et al., 2013). Their invasion is assisted by their traits of high reproductive output, parental care, omnivory, aggressive behaviour and tolerance to environmental parameters (e.g. Leunda et al., 2008; Novomeská et al., 2013). In the UK, however, they are not invasive, with only a single, persistent wild population believed to be present (Britton et al., 2010). The lag phase – the time period between the introduction of a species and an invasion developing – can be considerable for non-native fishes. For many species, its cessation requires a change in environmental conditions and/or the provision of a new dispersal opportunity (Fausch, 2007).

The aim of this study was to assess the reproductive ecology and diet of *A. melas* in UK conditions through assessment of this persistent population. As there was only one population available, the plasticity of their traits in UK conditions could not be assessed; however, the study results are compared with their populations elsewhere. An assessment is then made to identify whether these aspects of their ecology are inhibiting their invasion of UK freshwaters. Note that Novomeská et al. (2013) recently concluded that morphological plasticity was not a factor affecting their invasive ability and so is not considered here.

Materials and methods

This UK population is present in a 2 ha pond utilised for catch-and-release angling (N51°42′21″; E0°10′37″) and was introduced at least 30 years ago. Samples were collected monthly between May and December 2013 using 10 fish traps (1.2 × 0.5 × 0.3 m; 3 mm mesh size) with pelletized bait (21 mm diameter) and a 24 h soak. Following lifting, all fish were then removed from the traps. The *A. melas* were euthanized (overdose of MS-222) and transferred to the laboratory on ice. In the laboratory, the fish were counted, measured (total length, nearest mm) and weighed (to 0.1 g). After dissection, mature individuals were identified by macroscopic examination of the gonads, with the gonads then removed and weighed (0.1 g). These data enabled calculation of length at maturity from the percentage of mature fish in each 20 mm length class using the formula of DeMaster (1978), as modified by Trippel and Harvey (1987). For mature females (as identified through ripe oocytes being present in the ovary), temporal variation in gonad weight was analysed in ANCOVA, where fish length was the co-variate and month was the main effect. This provided the estimated marginal means of gonad weight per month, adjusted for fish weight, and the significance of their differences according to pairwise comparisons with Bonferroni adjustment for multiple comparisons. These were compared to mean water temperatures calculated from daily measurements via a temperature logger.

The *Ameiurus melas* diet was assessed by stable isotope analysis (SIA) using values of $\delta^{15}N$ as an indicator of trophic level and $\delta^{13}C$ as an indicator of energy source (Cucherousset et al., 2012). This was completed using samples of dorsal muscle from 30 randomly selected *A. melas* from July, their putative food resources (in triplicate samples, where possible) and samples of *Rutilus rutilus* (n = 30). The mean length of *A. melas* used in SIA was 165 ± 5 mm (range 130 to 220 mm), and 118 ± 5 mm (range 88 to 168 mm) in *R. rutilus*. All samples were dried at 50°C for 48 h before being analysed at the Cornell Isotope Laboratory for analysis (Cornell University, New York, USA). Data analysis initially examined the trophic relationship between *A. melas* and *R. rutilus* using standard ellipse areas (SEA_c) in the package 'siar' (Jackson et al., 2011, 2012) in the 'R' computing programme (R Core Team, 2013). These ellipses are based on the distribution of individuals in isotopic space as an estimate of each species' core trophic niche (Jackson et al., 2011). To then predict the diet composition of *A. melas*, isotope-mixing models were used. The Bayesian mixing model 'siarsolo' (Parnell et al., 2010) was used with *R. rutilus* (n = 30) and invertebrates (Hirudinea, Chironomidae, Asellidae, Gammaridae, Ephemeroptera and Odonata; n = 10) used as putative resources for each *A. melas* individual. Fractionation factors

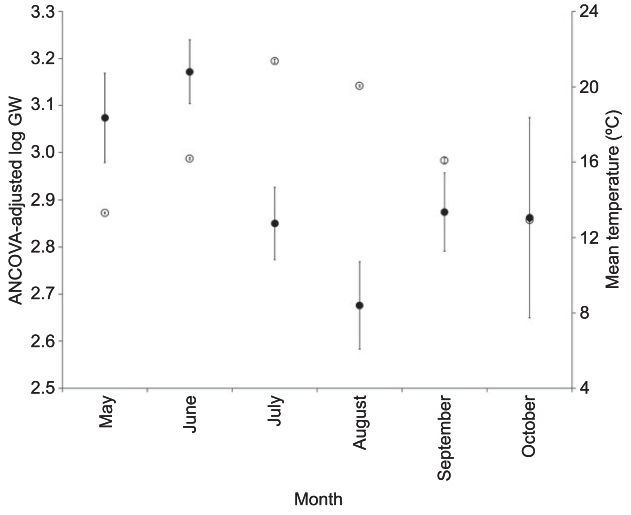

Fig. 1. Gonad development of female *Ameiurus melas* by month (solid circles), based on gonad weight adjusted for fish length in an analysis of covariance, versus mean water temperature for that month (hollow circles). Error bars = standard error; * = significant difference in gonadal development in that month relative to preceding months (pairwise comparisons with Bonferroni adjustment, $P < 0.05$).

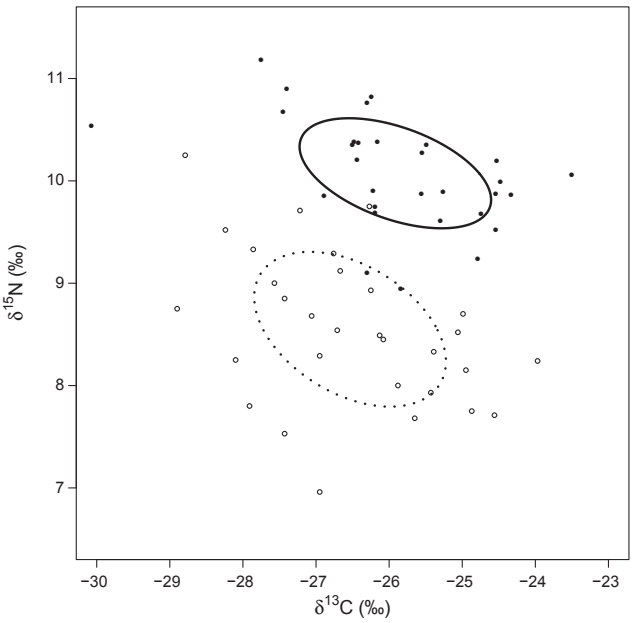

Fig. 2. Isotopic bi-plot of δ^{15}N and δ^{13}C. Symbols = individual bullhead (solid) and roach (hollow). Ellipses enclose the core niche width (SEAc) of bullhead (solid line) and roach (dashed line).

were 1.0 ± 1.0 ‰ for δ^{13}C, and 3.4 ± 1.0 ‰ for δ^{15}N. The SIA data were complemented by gut content analysis (GCA) of fish captured in June, July and August. The contents of the fish stomachs were removed and identified under low power microscopy (×10 to ×30). Identification of macro-invertebrates was to at least family level; where fish scales were encountered, it was assumed that the *A. melas* had consumed one fish. The traps were soaked for 24 h rather than 12 h due

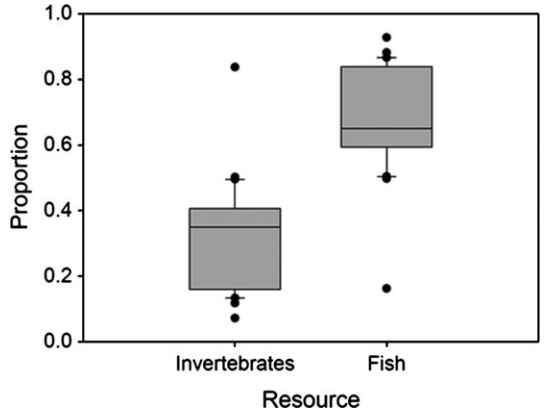

Fig. 3. Proportional contributions of macro-invertebrates ('Invertebrate') and fish resources ('Fish') to the individual bullhead (n = 30) diet based on the mixing model, Siarsolo. Each box encloses the 25th and 75th percentile; central line = the median. Error bars = the 10th and 90th percentile; symbols = outliers.

to logistical and access issues, thus there was a possibility that some ingested items had been digested or regurgitated. Therefore, analysis was restricted to the frequency of occurrence of each prey item, defined as the percentage of stomachs in which it occurred. Outputs were combined across the months, as no temporal variation was evident.

Results

The fish captured were between 46 and 208 mm, with the majority 100 to 180 mm (86%). No fish < 50 mm were captured before September, suggesting that these fish were young-of-the-year. The sex ratio across all samples was 1F:1.03M, and not significantly different from 1:1 ($\chi^2 = 0.06$, $P = 0.81$). Length at maturity was 100.4 mm (combined sexes). The effect of month on female gonadal development was significant ($F_{5,65} = 4.58$; $P < 0.01$), with pairwise comparisons revealing that the mean gonad weight in July was significantly lower than in June ($P = 0.04$), but not from August to October ($P > 0.05$; Fig. 1).

The standard ellipse areas revealed that the trophic niches of *A. melas* (1.94 ‰^{2}) and *R. rutilus* (2.83 ‰^{2}) did not overlap in isotopic space, with *A. melas* feeding at a comparatively higher trophic position (Fig. 2). The mixing model predicted that fish contributed more to the *A. melas* long-term assimilated diet than did macro-invertebrates (Fig. 3), with this being independent of *A. melas* length. The majority of *A. melas* stomachs contained small amounts of plant material and Chironomidae (larvae and pupae), although fish were also relatively prominent (Table 1). The remaining invertebrate prey items all had frequency of occurrences below 16%.

Discussion

Our results suggest that a high proportion of *A. melas* spawned between the collection of the June and July samples, a period coincident with the highest water temperatures of the study period (mean temp in July: 21.4 ± 0.30°C). It was also coincident with a relatively high mean air temp of

Table 1
Frequency of occurrence (%Fi) of prey items in stomachs of 80 *Ameiurus melas* captured between June and August 2013

Prey item	%Fi
Inorganic matter	34.0
Plant material	98.0
Fish	30.0
Chironomidae (larva)	72.0
Chironomidae (pupa)	18.0
Ceratopogonidae larva	2.0
Trichoptera larva	6.0
Coleoptera	6.0
Hydracarina	2.0
Gammaridae	10.0
Isopoda	16.0
Ostracoda	2.0
Branchiura	2.0
Gastropoda (Physidae)	4.0
Gastropoda (Planorbidae)	2.0
Gastropoda (Hydrobiidae)	2.0
Terrestrial insects	6.0

18.3°C (Central England Temperature; Meteorological Office, 2013). In its native range, *A. melas* spawning tends to occur at temperatures of 21°C (Dennison and Bulkley, 1972), whilst in their invasive range, Novomeská and Kováč (2009) suggested spawning in Slovakian oxbow lakes occurs at 18 to 22°C. Thus, temperature was not an inhibitor of *A. melas* reproductive success during our study period and so is unlikely to explain their inability to be invasive in the UK more generally.

The apparent importance of invertebrates in GCA, especially chironomids, is consistent with other GCA studies of invasive *A. melas* with, for example, Leunda et al. (2008) revealing a similar pattern in Iberian populations. The partial discrepancy in the outputs of the dietary analysis between SIA and GCA is also consistent with the study on the diet of *Lepomis gibbosus* by Locke et al. (2013), who found little association in diet composition when compared using three methods, including GCA and SIA. They argued this was due to gut contents providing only a dietary snap-shot in which slowly digested items (e.g. plant material) might be over-represented, whereas SIA reflects an assimilated diet over a longer time period, i.e. many items found during GCA might not contribute to an assimilated diet.

In conclusion, there was little evidence to suggest that the inability of *A. melas* to be invasive in the UK was related to insufficient summer temperatures for their reproduction, with their integration into the food web at a relatively high trophic level suggesting that they also have access to ample food resources to facilitate their persistence. Thus, the continuation of their lag phase in the UK appears more related to their lack of dispersal opportunities from this single population in the wild than through ecological constraints. Correspondingly, should individuals from this population disperse in the future, then invasive populations might subsequently develop.

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