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Research Article

A non-native snakehead fish in British Columbia, Canada: capture, genetics, isotopes, and policy consequences

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Abstract

In June 2012 a single non-native snakehead fish was captured by local officials in a small pond within an urban park in Burnaby, British Columbia. This single snakehead fish garnered significant attention in the local and national media. DNA analysis determined it to be a blotched snakehead (*Channa maculata*) or possibly a hybrid; a warm water species native to China and Vietnam which is commonly sold in the live food fish trade, and occasionally kept by hobbyists. By collecting prey items from the pond and snakehead specimens from fish markets we used a novel stable isotope approach to estimate how long it had been since the snakehead had been released into the pond. Using a diet-switching tissue turnover model, we estimated that the snakehead was in the pond between 33 and 93 days. Subsequently, provincial legislation was amended to ban all species of snakehead fish, as well as numerous other potentially invasive fish and invertebrate species.

Key words: stable isotope analysis; tissue turnover model; failed invasion; invasion pathways; Fraser River

Introduction

Invasive species are generally studied only after they have become established and spread (Zenni and Nuñez 2013), leaving an absence of studies focusing on the first phases of invasion: transport and introduction (Kolar and Lodge 2001). Once a species has proliferated and caught the attention of managers or scientists, it can be difficult to reconstruct critical information regarding key initial phases of introduction (but see Cadien and Ranasinghe 2001). Furthermore, these initial phases of invasion tend to filter out the vast majority of most potential exotic species from those that could be invasive. Only about 1% of new non-native imports become invasive (Williamson 2006). Studies of potentially harmful non-native species in the initial phases of invasion, including species that fail to establish and spread, can thus advance our understanding of the processes that underpin potential invaders (Zenni and Nuñez 2013).

Further understanding of invasive pathways could be particularly useful for preventing the release and establishment of species identified as emerging threats such as the snakehead group of fish (Family: Channidae) (Courtenay and Williams 2004; Herborg et al. 2007). This family consists of 29 species of two genera with home ranges in Southeast Asia, Russia and Africa (Courtenay and Williams 2004). To date there have been numerous introductions in North America, including populations of blotched snakeheads (*Channa maculata* Lacepède, 1801)

in Hawaii, bullseye snakehead (*C. marulius* Hamilton, 1822) in Florida (Courtenay and Williams 2004) and northern snakehead (*C. argus* Cantor, 1842) in the Potomac River in the mid-Atlantic United States (Odenkirk and Owens 2007). These multiple introductions likely occurred in part because snakeheads are propagated in aquaculture facilities and occur in the live fish trade, both for consumption and as aquarium pets (Courtenay and Williams 2004), and are particularly prized for their purported medicinal properties (Lee and Ng 1991). These examples of rapid establishment of snakeheads highlight that these fish can flourish in a wide range of environmental conditions—they tolerate hypoxic conditions, have the ability to perform aerial respiration, and some species are capable of making small overland movements and withstanding days out of water if kept hydrated (Courtenay and Williams 2004). Furthermore, snakeheads could have broad ecological effects if they flourish. Snakeheads are predatory fishes that can eat a diversity of prey (Odenkirk and Owens 2007) and can reach large sizes (Courtenay and Williams 2004). In the Potomac River, fisheries managers predict that continued uncontrolled range expansion of the northern snakehead population could lead to up to a 35% population reduction of a valuable largemouth bass (*Micropterus salmoides* Lacepède, 1802) recreational fishery (Love and Newhard 2012). Snakeheads continue to be a threat for invading freshwater systems in North America due to the possibility of multiple introductions from the aquarium and live-food fish trade and their ability to quickly establish a flourishing population that impacts local ecosystems (Courtenay and Williams 2004; Herborg et al. 2007). This has led to a federal ban on live importation and interstate transport in the United States and a ban on live possession in 26 US states and Ontario, Canada (Courtenay and Williams 2004; Herborg et al. 2007).

Snakeheads were identified as an ongoing threat to Canada's freshwater ecosystems by the Fisheries and Oceans Canada (Cudmore and Mandrake 2005). A single large snakehead was discovered in a small urban pond in Burnaby, B.C., in spring 2012 and subsequently removed, attracting media attention from across Canada. Here we report on this noteworthy failed invasion, presenting what we learned from the fish specimen using genetics and a novel stable isotope approach, and discuss the policy consequences of this single fish introduction.

Methods

Detection and capture

Initial detection of a snakehead was publicised by a private individual who observed and videotaped the fish swimming near the surface of the pond and then posted a video on the internet on May 11th, 2012. The posting led to local, regional and national media coverage starting on May 15th, 2012. The fish was videoed at Lower Pond (49°13'23.57"N, 123°01'09.68"W), a shallow isolated man-made pond (0.86 ha in size) in a small urban park, called Central Park, in Burnaby, BC. The pond drains into the lower Fraser River via a small outflow channel. The first site assessment of the pond was conducted on May 23rd, 2012 to determine the physical properties of the pond, the species present, and possible capture methods. Backpack electroshockers and different beach seine nets (63 mm and 2.5 cm mesh sizes) were tested to determine their effectiveness in this particular water body. Backpack electrofishing equipment was ineffective as all larger fish avoided the increasing strength of the electrical field. Boat-based electrofishing may have been more effective; however, the pond was shallow and had no boat access, so this approach was not pursued. For the main capture effort on June 8th, 2012 the pond was subdivided into 4 sections by stop nets (0.63 cm mesh size) and due to the inflow into the pond coming from the municipal water supply, the water level was reduced to 1 m across the pond. Then a seine net (2.5 cm mesh size) was run from shore to one of the stop nets, and then back on the adjacent stop net, encircling the whole quarter section of the pond. It was then drawn slowly to shore ensuring that the net did not lift off the bottom. Using this process the whole area of the pond was seined, while maintaining separations of the different sections using the stop nets. During the sectional seining (2.5 cm mesh size) the snakehead and several other potential prey fish species collected in the pond were euthanized using buffered Tricaine Methanosulfonate (MS222) according to American Veterinarian Medical Association procedures, and retained for further analyses. The single adult snakehead fish was collected and euthanized, measuring 690 mm in total length and weighing 3700 g. No other snakeheads were captured. While we do not have data on water temperatures, as a surrogate the average air temperature, as reported on the Government of Canada's public climate database, was 3.9°C

Table 1. Summary of all fish captured from Lower Pond (49°13'23.57"N, 123°01'9.68"W) located in an urban park in Burnaby, B.C. on June 8th, 2012. The fish noted as large are not considered potential prey items for the snakehead. We did not differentiate between small carp and goldfish.

Fish	Number captured
Carp (*Cyprinus carpio*)—large	65
Carp (C. carpio) or Goldfish (*Carassius auratus*)—small	50
Fathead Minnow (*Pimephales promelas*)	49
Brown Bullhead (*Ameiurus nebulous*)	78

in Burnaby during March 2012. The removal effort consisted of approximately 300 hours of staff time.

Identification and genetic analyses

We applied a recently developed DNA barcoding library for snakeheads as well as fin ray and scale counts to identify this cryptic species (Serrao and Hanner in review). As a tool for species identification and discovery, DNA barcoding uses a standardized ~650 base-pair segment of the mitochondrial 5' cytochrome *c* oxidase subunit I (COI) gene region (Hebert et al. 2003). The Fish Barcode of Life (FISH-BOL) campaign was launched to create a barcode reference library for all fishes and has resulted in a broad coverage for a variety of species, including snakeheads. This database (BOLD; Ratnasingham and Hebert 2007) can be queried to infer the identification of an unknown query sequence (Ward et al. 2009).

DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (QIAGEN), with two exceptions (see Serrao et al., in review). The polymerase chain reaction (PCR) thermocycling conditions were an initial hot start of 94°C for 2 min, 25 cycles of [denaturation at 94°C for 30 s, annealing at 52°C for 40 s and extension at 72°C for 1 min], with a final extension at 72°C for 10 min using the universal fish cocktail primers (Ivanova et al. 2007). The PCR products were visualised using 2% agarose gel (E-Gel96 Pre-cast Agarose Electrophoresis), after which they were bidirectionally sequenced and run on an ABI 3730 capillary sequencer (Applied Biosystems). The thermocycling profile was an initial hot start 96°C for 2 min, followed by 30 cycles of [denaturation at 96°C for 30 s, annealing at 55°C for 15 s, and an extension at 60°C for 4 min]. Sequencher 4.05 (GeneCodes) was used to trim primers, assemble and manually edit bidirectional contigs from raw trace files and sequences were compared against the snakehead reference library on BOLD (see Braid et al. 2012 for further method details). Fin ray counts from the pectoral, dorsal and anal fins along with scale counts were done and compared with snakehead species taxonomic guidelines from Courtenay et al. (2004).

Stable Isotopes

We developed a novel application of stable isotopes to estimate how long the snakehead was in the pond. Different tissues have different tissue-specific turnover rates. After a diet switch, tissues take different amounts of time to represent the new diet isotopic signature (Phillips and Eldridge 2006). Estimating the timing of diet switch necessitates quantifying the isotopes of the consumer and the isotope signature of the original and new food sources (Phillips and Eldridge 2006; Buchheister and Latour 2010). Accordingly, samples of muscle, fin, heart and liver tissue were removed from the snakehead specimen for stable isotope analysis and frozen. Numerous other fish were present in the pond (Table 1), all of which were non-native. Samples were retained for stable isotope analyses from goldfish (N = 1; *Carassius auratus auratus* Linnaeus, 1758), brown bullhead (N = 1; *Ameiurus nebulous* Lesueuer, 1819*)* and fathead minnow (N = 5; *Pimephales promelas* Rafinesque, 1820). These fish were frozen, then later thawed and muscle and fin tissues sampled. Liver tissue was sampled for only the goldfish and bullhead because minnows were too small to effectively extract liver tissue. We focused on muscle tissue from all of these fish as representing potential prey.

To estimate the pre-introduction isotopic signature for the snakehead we sought to purchase additional specimens from potential sources of snakeheads. As there are many different sources from which snakeheads can be acquired we hoped that by sampling multiple sources we would be more likely to find a source which would potentially reflect the pre-introduction isotopic signatures of the snakehead captured in

the pond. Five imported snakeheads came from a supplier in Richmond, BC (purportedly wild caught in Vietnam and transported frozen). These imported snakeheads measured an average 409 mm long and weighed an average of 698 g. Three other frozen snakeheads, which will be referred to as the market snakeheads, were purchased from the shelf of a supermarket in Vancouver, BC, where live snakeheads were available in the past. Snakehead species are listed for sale by Canadian internet aquarium sites but we were unable to obtain any for analysis, possibly as a result of the recent media attention. Muscle, fin, heart and liver samples from the imported snakeheads, and muscle and fin samples from the market snakeheads as they were being used for a concurrent study reliant on intact morphology, were removed for stable isotope analysis.

All samples for stable isotope analysis were thawed, rinsed with deionized water, and dried for 48 hours at 60°C. All muscle, heart and liver samples were then separately homogenized into a powder using a mortar and pestle. Pieces of dried fin and powder were weighed in 5 × 9-mm tin capsules until targeted weights (1.0 ± 0.10 mg) were achieved. Samples were analyzed at the University of California Davis Stable Isotope Facility for carbon and nitrogen stable isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Values are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and air for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), respectively, which are commonly used elements in food web studies (Buchheister and Latour 2010).

Tissue turnover model

We estimated time in the pond by calculating time since diet switch for our snakehead using the $\delta^{13}C$ stable isotope data and a single compartment discrimination factor model. This model uses prey isotopes, discrimination factors and equilibrium tissue values and assumes a single pool through which isotopes turnover at a single rate (Phillips and Eldridge 2006; Heady and Moore 2013). We assumed a $\delta^{13}C$ discrimination factor between diet and fish tissue of 0.80±1.13%, the average across 42 studies excluding herbivores from a review by Vander Zanden and Rasmussen (2001) as there is no published value specific to snakeheads. We used a value for muscle tissue half-life of 135±32.9 days which represented an average from a study of adult bluegill (*Lepomis macrochirus* Rafinesque, 1819), largemouth bass, and yellow perch (*Perca flavescens* Mitchill, 1814) (Weidel et al. 2011). This value is very similar to the 138 days reported from a study on adult gag (*Mycteroperca microlepis* Goode and Bean, 1879), a marine piscivore (Nelson et al. 2011). While no studies to date report half-lives for adult fin tissue, one study by Suzuki et al. (2005) reported the difference in turnover rates between muscle and fin tissue in juvenile Japanese temperate bass (*Lateolabrax japonicas* Cuvier and Valenciennes, 1828). We used the ratio of the length of fin to muscle turnover rates determined by Suzuki et al. (2005) multiplied by our estimate for muscle tissue to generate an estimate of fin turnover half-life of 165±40.2 days. We did not find a published tissue turnover rate for adult fish heart tissue, so did not include this in the analysis. Liver tissue has a rapid turnover (approximately 10–20 days; Buchheister and Latour 2010), and it appeared that the pond snakehead liver tissue was in equilibrium with the pond's isotope signature, so could not be used in the turnover model (see results and discussion). We used the isotopic signature of the market snakeheads to represent the pre-switch signature based on the isotope space. While not necessarily from the same origin it appears the market snakeheads had a similar past diet to that of the pond snakehead, which could be attributable to being held in captivity and being fed similar diets.

We used single-tissue clocks using equilibrium tissue values, prey isotopes, and a single discrimination factor to solve each tissue turnover model for the time since diet switch (T_{est}) as described in Heady and Moore (2013). This method requires the measured isotope signature for each tissue (δX_{Iso}), the estimated isotope signature of the origin population (δX_{Pre}), and the estimated isotope signature of the new diet (δX_{Post}), as well as our estimates derived from the literature of tissue specific turnover rates (λ) and discrimination factor (Δ). For δX_{Post}, we used the muscle isotope signature of all prey fish from the pond. We were then able to use our estimates of each parameter to model the residence time based on each tissue using the following equation from Heady and Moore (2013):

$$T_{est} = \lambda \times \log\left((\delta X_{Post} + \Delta - \delta X_{Iso}) / (\delta X_{Post} - \delta X_{Pre})\right)$$

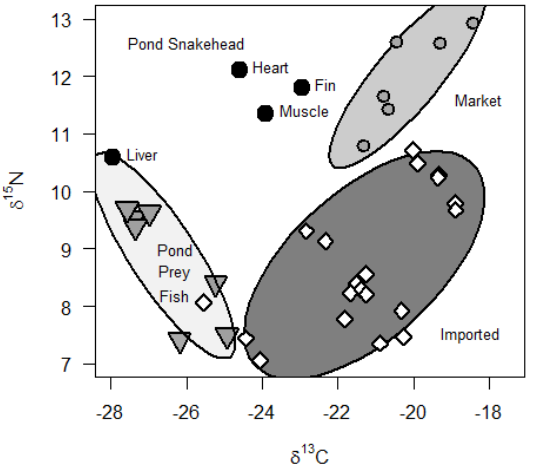

Figure 1. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) from the pond snakehead, its sources, and its new prey. Individual values obtained from the samples are shown for the pond prey fish (triangles), market blotched snakeheads (circles) and imported blotched snakeheads (diamonds). The large black dots represent the average value of two samples for each of the different tissues sampled, with the exception of three samples from muscle tissue from the blotched snakehead captured in the pond. The ellipses represent 75% confidence ellipses around the values for each of the other groups. The pond snakehead and prey fish were captured in Lower Pond (49°13'23.57"N, 123°01'09.68"W) located in an urban park in Burnaby, B.C. on June 8th, 2012. The market and imported snakeheads were purchased frozen in Vancouver, B.C. in fall 2012.

We propagated uncertainty in parameter values by bootstrapping all parameters with standard deviations with 100,000 iterations. The time estimate was the average of individual model estimates calculated for muscle and fin tissue. All statistical analyses were performed with the R statistical package (R Development Core Team 2012).

Results and discussion

The snakehead from the pond as well as the imported and market snakeheads were all blotched snakehead (*C. maculata*). All specimens showed 100% sequence identity with the *C. maculata* reference sequences on BOLD. It should be noted that DNA barcoding cannot differentiate between the hybrid and *C. maculata* (Zhu et al. 2012); thus this technique cannot rule out the possibility that specimens could represent hybrids between *C. maculata* (female) and *C. argus* (male). However, fin ray and scale counts also indicated that our specimen was *C. maculata* (G. Hanke personal communication) following the guidelines of Courtenay et al. (2004), which reduced the likelihood that it was a hybrid. One known established population of *C. maculata* exists within North American (Courtenay and Williams 2004). According to a review by Courtenay and Williams (2004), the probable mechanism of introduction for *C. maculata* in North America is release of live food fish, as they are common in the live food trade yet only occasionally available in the aquarium trade. Since only one individual was discovered, it seems unlikely this was an attempt to seed a population for harvest, but perhaps it may have been a ceremonial or a hobbyist release after the fish became too large (Severinghaus and Chi 1999). While this blotched snakehead has previously received less attention as a potential invader than the northern snakehead due to its lower thermal tolerance for cooler water temperatures (Herborg et al. 2007), blotched snakeheads have been widely introduced and are generally poorly studied. They have been introduced in Japan and have become established far north of their native ranges so their thermal limits may be less restricted than previously thought (Courtenay and Williams 2004). Furthermore, ongoing climatic change may shift thermal boundaries for potential invasive species such as the blotched snakeheads.

Stable isotope analysis provided insight into the invasive history of the pond snakehead. The sources of snakeheads (both market and import) were more enriched in $\delta^{13}C$ than potential prey items (Figure 1). Introduced pond snakehead tissues had isotope signatures that were in between potential prey items and market or imported snakeheads, although it depended on tissue (Figure 1). The isotopic signature of the pond snakehead's liver, which has the fastest turnover of the measured tissues (Suzuki et al. 2005), was similar to that of the prey items in the pond, indicating that the liver tissue had approached equilibration with the new pond isotope signatures. The longer turnover tissues of the pond snakehead, such as muscle, fin and heart, were intermediate in $\delta^{13}C$, between the pond prey and the proposed source snakeheads from the market (Figure 1). Thus tissues had begun to incorporate the signature of the pond prey items while still partially representing their pre-introduction diet.

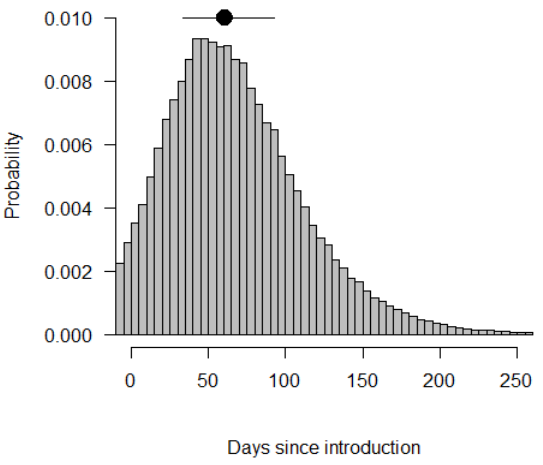

Figure 2. Estimate of time (days) since introduction for the blotched snakehead captured in a small pond (49°13'23.57"N, 123°01'09.68"W) in an urban park in Burnaby, B.C. on June 8th, 2012. Shown is the distribution of time estimates, based on 100,000 bootstrapping iterations using a single compartment tissue turnover model from both muscle and fin tissue $\delta^{13}C$ values. Point and lines represent the median estimate and 25 and 75% confidence intervals.

The tissue turnover model suggested the pond snakehead was introduced one to three months prior to capture (median estimate 65.6 days with first and third quartile estimates of 33.1 and 92.3 days; Figure 2). Our estimates are based on previous estimates of tissue turnover, which may be influenced by temperature or other factors. Our results suggest the snakehead was released during late winter or early spring 2012 and was subsequently captured on June 8, 2012. The lower limit on this time frame is supported by the timeline of the video and subsequent capture; 26 days had elapsed between the posting of the video sighting the fish and its eventual capture indicating that the snakehead had been in the pond at least that long.

This was a relatively quick detection of an introduction event. When northern snakeheads were discovered by anglers in a Crofton, Maryland pond in 2002, it was later revealed by the individual who released them that they had been present and unnoticed for two years (Dolan 2003). The invasive northern snakehead population in the Potomac River in Virginia expanded from a catch per unit effort of 0.2 fish/h in 2004 to 6.1 fish/h in 2006 (Odenkirk and Owens 2007), thus snakeheads can proliferate quickly and undetected. First noted in 2004, no estimate exists of when they were first introduced to the Potomac River (Orrell and Weigt 2005), limiting the ability of managers to infer their original source. Quick detection in our case was likely related to the pond being quite small (0.86 ha) and located in a busy urban park. While this introduction was quickly detected and did not result in an invasive population, it is still serious cause for concern. For example, if a pair of northern snakeheads had been released into the pond, then they could have established and spread relatively quickly. The pond is located within the Fraser River watershed; thus it could have had led to further spread with potential negative consequences for one of the most productive salmon producing rivers in North America. Preventing the introduction and spread of snakeheads will depend on angler awareness and laws prohibiting possession of live snakeheads.

The detection and capture of this single snakehead was associated with subsequent changes to invasive species legislation in the province of British Columbia. Due to the tremendous media attention generated by this story, the government of British Columbia passed a legislative amendment to ban possession, transport, and breeding of all members of the family Chaniidae in December 2012. This amendment also banned seven species of the family Cyprinidae, five species of the family Gobiidae, all species of the family Ictaluridae and three species of dreissenid mussels. Despite the provincial ban, there is still threat of introduction of snakeheads via illegal possession and trade. In Ontario, Canada snakeheads have been banned since 2007. However in November 2012, a pet dealer from Markham, Ontario was arrested for selling 228 live snakeheads to an undercover officer. Similarly, in April 2013 a snakehead was spotted in a pond in Central Park, New York, USA, where possession has been illegal for over a decade. Given continued threat of snakehead invasion via illegal trade, continued monitoring, public education and rapid response planning are warranted.

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