

**UNIWERSYTET GDAŃSKI**  
**WYDZIAŁ OCEANOGRAFII I GEOGRAFII**

**Wpływ wybranych warunków środowiskowych na  
przeżywalność, tempo wzrostu oraz kanibalizm wczesnych  
stadiów rozwojowych szczupaka (*Esox lucius*) – badania  
eksperymentalne**

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The effect of selected environmental variables on the survival, growth rate, and cannibalism of the early life stages of pike (*Esox lucius*) – experimental study



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## Podziękowania

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## Streszczenie

W ciągu ostatnich kilku dekad dostępność tarlisk dla populacji anadromicznej szczupaka (*Esox lucius* Linnaeus, 1758) w rejonie Morza Bałtyckiego została znacznie zredukowana w wyniku oddziaływania czynników antropogenicznych, takich jak regulacja rzek, prace melioracyjne oraz budowa przepompowni. Dodatkowo brak występowania w niektórych obszarach geograficznych szczupaka rozradzającego się w wodach słonych spowodował drastyczny spadek liczebności populacji szczupaków na tych obszarach. W Zatoce Puckiej istnienie populacji szczupaka jest obecnie podtrzymywane głównie poprzez zarybienia narybkiem pochodzącym z podchowu w ośrodkach hodowlanych, w oparciu o tarlaki „słodkowodne”. Ten stan rzeczy, w kontekście potrzeby wypracowania planów poprawy sytuacji, wymusza wręcz konieczność lepszego poznania ekologii gatunku, zwłaszcza w okresie wczesnego rozwoju - procesy zachodzące w okresie rozwoju embrionalnego, larwalnego oraz juvenilnego mogą lokalnie skutkować nawet 100% śmiertelnością.

Wyniki przedstawione w niniejszej pracy uzyskano w ramach dwóch eksperymentów przeprowadzonych w oparciu o system akwariów z zamkniętym obiegiem wody: eksperyment nr 1 dotyczył wpływu zasolenia na rozwój embrionalny szczupaka, natomiast eksperyment nr 2 wpływu temperatury wody na tempo wzrostu larw, intensywność zjawiska kanibalizmu oraz wzrost otolitów stadiów młodocianych szczupaka w pierwszym miesiącu życia. Analizowano również występowanie asymetrii fluktuacyjnej otolitów. Podczas eksperymentu nr 1 określono stadium rozwojowe dla 3500 ziaren ikry. Podczas eksperymentu nr 2 analizie poddano 810 osobników oraz 71 par kanibalistycznych. Zmierzono wielkość 1440 otolitów (długość i szerokość strzałek i kamyczków) do analizy ich tempa wzrostu oraz 284 otolitów (powierzchnia, długość i szerokość strzałek) do analizy fluktuacyjnej asymetrii otolitów pobranych od par kanibalistycznych.

Przeprowadzone badania pozwoliły na wykazanie że:

1. Rozwój ikry jest zależny od warunków zasoleniowych panujących podczas zapłodnienia – sukces wylęgu w 0 PSU wynosił 89,7%, w 4 PSU 19,7%, a w 7 PSU 0%. Jeśli natomiast samo zapłodnienie następowało w wodzie słodkiej,



zasolenie na poziomie 4 oraz 7 PSU nie wpływa negatywnie na późniejszy (po godzinie od zapłodnienia) rozwój embrionalny i sukces wylęgu.

2. Temperatura wody w zakresie od 10 do 22°C ma pozytywny wpływ na tempo wzrostu larw i wczesnego narybku szczupaka – optimum dla wzrostu jest więc równe lub większe od 22°C.
3. Temperatura wody ma istotny wpływ na zjawisko kanibalizmu w okresie larwalnym i wczesno-juwenilnym. W wyższej temperaturze rozpoczęcie ataków kanibalistycznych następuje szybciej (w 22°C już po 5 dniach od wylęgu), a ich częstotliwość jest większa. Temperatura nie miała natomiast wpływu na stosunek wielkości ofiary do wielkości kanibala. Najmniejsze różnice w wielkości pomiędzy ofiarą a kanibalem wynosiły zaledwie 0,2 mm, co znaczy że wielkość ofiary była praktycznie taka sama, jak wielkość kanibala.
4. Różnica w asymetrii fluktuacyjnej wielkości otolitów (FA) nie występuje pomiędzy kanibalami a ofiarami, zarówno dla strzałek, jak i kamyczków. Jeśli nawet przynależność do grupy ofiar w populacji jest związane ze stresem, nie znajduje to odzwierciedlenia w FA.
5. Tempo wzrostu otolitów, a więc również szerokość odkładanych na otolitech przyrostów dobowych, jest ściśle powiązana z tempem wzrostu larw i narybku szczupaka.

Podstawowym sposobem na odbudowę populacji szczupaka w Zatoce Puckiej jest przywrócenie miejsc tarliskowych dla tego gatunku. Uwzględniając powyżej opisane wyniki, należy zwrócić uwagę podczas tych prac zarówno na dostępność pokarmu dla żerującego narybku szczupaka, jak i na zapewnienie mu odpowiedniej przestrzeni w celu zminimalizowania kanibalizmu. Zwłaszcza, że w wysokich temperaturach płytkich rozlewisk tempo wzrostu larw i narybku szczupaka jest szybkie i w krótkim czasie prowadzi do pojawienia się dużych różnic w wielkości pomiędzy osobnikami z tego samego terminu wylęgu, co sprzyja intensywnemu kanibalizmowi. Ikra składana w wodzie słodkiej na przybrzeżnych rozlewiskach jest o tyle bezpieczna, że napływ wód słonych nie powinien wyrządzić szkód, jeśli tylko ikra została zapłodniona i rozwój embrionalny już się rozpoczął. Planując w przyszłości badania wczesnych stadiów rozwojowych szczupaka w warunkach naturalnych, warto rozważyć możliwość

uzyskania niektórych informacji na podstawie analizy mikrostruktury i wielkości otolitów, gdyż okazały się one spełniać podstawowe założenia metodyczne i mogą stanowić źródło wiedzy o wieku i tempie wzrostu larw i narybku szczupaka.

## Abstract

Over the past few decades the availability of spawning grounds for anadromous populations of northern pike (*Esox lucius* Linnaeus, 1758) in the Baltic Sea region has been reduced significantly by the effects of anthropogenic factors such as river regulation, drainage works, and pumping station construction. Additionally, pike that spawn in marine waters no longer occur in some geographic areas, which has led to drastic declines in pike populations in them. The pike population in the Puck Lagoon is currently mostly maintained by stocking with fry produced in hatcheries using “freshwater” spawners. Consequently, developing plans to improve the situation of northern pike requires a better understanding of the ecology of this species particularly in the early developmental stages and of the processes that occur during embryonic, larval, and juvenile development that can lead to local mortality rates as high as 100%.

The results presented in this article are those obtained during two experiments conducted in recirculating aquaculture systems: experiment 1 focused on the influence of salinity on pike larval development, and experiment 2 focused on the influence of water temperature on larval growth rates, the intensification of cannibalism, and otolith growth in pike juveniles in the first month of life. The occurrence of otolith fluctuating asymmetry was also analyzed. During experiment 1, the developmental stages of 3,500 eggs was identified, while during experiment 2, 810 specimens and 71 cannibalistic pairs were analyzed. The size of 1,440 otoliths was measured (sagittae and lapilli lengths and widths) to analyze their growth rates, and 284 otoliths (sagittae surface areas, lengths, and widths) to analyze fluctuating asymmetry in otolith samples from cannibalistic pairs.

The studies revealed the following:

1. Larval development depends on the prevailing salinity conditions during fertilization, and hatching success was 89.7% at 0 PSU, 19.7% at 4 PSU, and 0% at 7 PSU. However, if fertilization occurred in freshwater, one hour after fertilization salinities of 4 and 7 PSU did not negatively influence further embryonic development or hatching success.

2. Water temperatures within the range of 10 to 22°C had a positive influence on pike larval and early fry growth rates; thus the optimum temperature for growth was 22°C or higher.
3. Water temperature significantly influenced the occurrence of cannibalism during the larval and early juvenile stages. At higher temperatures, cannibalistic attacks occurred sooner (five days following hatching at 22°C) and their frequency was greater. Water temperature did not influence the ratio of victim size to cannibal size. The smallest size difference between victims and cannibals was barely 0.2 mm, which indicates that the size of the victims and cannibals was practically the same.
4. Differences in the fluctuating asymmetry (FA) of otolith sizes did not occur between cannibals and their victims, and this pertained to both sagittae and lapilli. Even if belonging to the victim group within populations was associated with stress, this was not reflected in FA.
5. Otolith growth rates, and thus the widths of daily growth increments, was strictly linked with pike larval and fry growth rates.

The basic way to rebuild the pike population in the Puck Bay is to restore its spawning grounds. Considering the results described above, restoration work should focus on ensuring food availability for foraging pike fry and providing them with adequate space to minimize cannibalism. This is especially important since pike larval and fry growth rates are fast at the high temperatures of shallow wetland waters, and large size differences among individuals from the same hatch date appear very quickly, which can lead to intense cannibalism. Eggs deposited in fresh coastal wetland waters are safe because the influx of salt water should not cause damage as long as the eggs have been fertilized and embryonic development has already begun. When planning future studies on early developmental pike stages in natural conditions, it would be worth considering the possibility of obtaining some of the data from the analyses of the microstructures and sizes of otoliths since they fulfill basic methodological assumptions and could be sources of knowledge regarding pike larval and fry ages and growth rates.

## Lista publikacji stanowiących rozprawę doktorską

1. **Greszkiewicz Martyna**, Fey Dariusz P., Lejk Adam M., Zimak Michał (2022). The effect of salinity on the development of freshwater pike (*Esox lucius*) eggs in the context of drastic pike population decline in Puck Bay, Baltic Sea. Hydrobiologia <https://doi.org/10.1007/s10750-022-04893-x>

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Punktacja MEiN: 100

Wkład w powstanie publikacji: 65%

2. Fey Dariusz P., **Greszkiewicz Martyna** (2021). Effects of temperature on somatic growth, otolith growth, and uncoupling in the otolith to fish size relationship of larval northern pike, *Esox lucius* L. Fisheries Research 236, 105843. <https://doi.org/10.1016/j.fishres.2020.105843>

Impact factor (IF): 2,69

Punktacja MEiN: 100

Wkład w powstanie publikacji: 50%

3. **Greszkiewicz Martyna**, Fey Dariusz P. (2020). Positive temperature effects on the initiation and intensity of cannibalistic behaviour of larval pike, *Esox lucius* L. Is cannibalism reflected in otolith fluctuating asymmetry? Hydrobiologia 847, 3139–3152. <https://doi.org/10.1007/s10750-020-04328-5>

Impact factor (IF): 2,69

Punktacja MEiN: 100

Wkład w powstanie publikacji: 80%

## Lista pozostałych publikacji autora

1. Jakubowska Magdalena, **Greszkiewicz Martyna**, Fey Dariusz P., Otremba Zbigniew, Urban-Malinga Barbara, Andrulowicz Eugeniusz (2021). Effects of magnetic fields related to submarine power cables on the behaviour of larval rainbow trout (*Oncorhynchus mykiss*). *Marine and Freshwater Research* 72, 1196-1207. <https://doi.org/10.1071/MF20236>

Impact factor (IF): 2,07

Punktacja MEiN: 70

2. Fey Dariusz P., **Greszkiewicz Martyna**, Jakubowska Magdalena, Lejk Adam M., Otremba Zbigniew, Andrulowicz Eugeniusz, Urban-Malinga Barbara (2019). Otolith fluctuating asymmetry in larval trout, *Oncorhynchus mykiss* Walbaum, as an indication of organism bilateral instability affected by static and alternating magnetic fields. *Science of The Total Environment* 707, 2020, 135489. <https://doi.org/10.1016/j.scitotenv.2019.135489>

Impact factor (IF): 7,96

Punktacja MEiN: 200

3. Fey Dariusz P., Jakubowska Magdalena, **Greszkiewicz Martyna**, Andrulowicz Eugeniusz, Otremba Zbigniew, Urban-Malinga Barbara (2019). Are magnetic and electromagnetic fields of anthropogenic origin potential threats to early life stages of fish? *Aquatic Toxicology* 209, 150-158. <https://doi.org/10.1016/j.aquatox.2019.01.023>

Impact factor (IF): 4,96

Punktacja MEiN: 140

4. Stankevičiūtė Milda, Jakubowska Magdalena, Pažusienė Janina, Makaras Tomas, Otremba Zbigniew, Urban-Malinga Barbara, Fey Dariusz P., **Greszkiewicz**

**Martyna**, Sauliutė Gintarė, Baršienė Janina, Andruliewicz Eugeniusz (2019). Genotoxic and cytotoxic effects of 50 Hz 1 mT electromagnetic field on larval rainbow trout (*Oncorhynchus mykiss*), Baltic clam (*Limecola balthica*) and common ragworm (*Hediste diversicolor*). *Aquatic Toxicology* 208, 109-117. <https://doi.org/10.1016/j.aquatox.2018.12.023>

Impact factor (IF): 4,96

Punktacja MEiN: 140

5. Fey Dariusz P., Lejk Adam M., **Greszkiewicz Martyna** (2018). Daily deposition of growth increments in sagittae and lapilli of laboratory-reared larval northern pike (*Esox lucius*). *Fishery Bulletin NOAA* 116, 302–309. <https://doi.org/10.7755/FB.116.3-4.8>

Impact factor (IF): 1,0

Punktacja MEiN: 70

6. Fey Dariusz P., **Greszkiewicz Martyna**, Otremba Zbigniew, Andruliewicz Eugeniusz (2018). Effect of static magnetic field on the hatching success, growth, mortality, and yolk-sac absorption of larval Northern pike *Esox lucius*, *Science of The Total Environment* 647, 1239-1244. <https://doi.org/10.1016/j.scitotenv.2018.07.427>

Impact factor (IF): 7,96

Punktacja MEiN: 200

7. **Greszkiewicz Martyna**, Fey Dariusz P. (2018). Effect of Preservation in Formalin and Alcohol on the Growth Rate Estimates of Larval Northern Pike. *North American Journal of Fisheries Management* 38, 601-605. <https://doi.org/10.1002/nafm.10059>

Impact factor (IF): 1,42

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### Dane bibliometryczne – podsumowanie

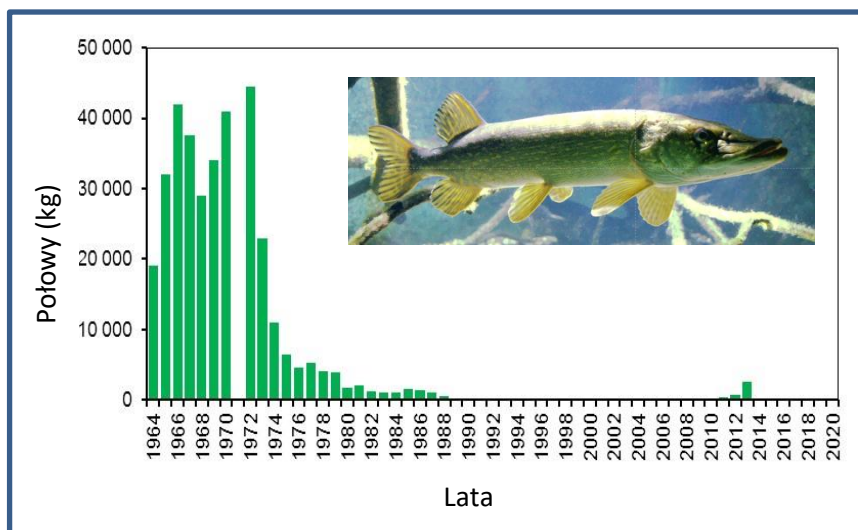
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Rozprawa doktorska	8,07	300	2
Pozostałe publikacje	30,33	890	63
<b>RAZEM</b>	<b>38,4</b>	<b>1190</b>	<b>65</b>



## Uzasadnienie podjętych badań

Szczupak jest jednym z najcenniejszych gatunków w gospodarce rybacko-wędkarskiej ryb drapieżnych, zarówno w jeziorach i rzekach, jak i w strefie przybrzeżnej niektórych rejonów Bałtyku. Jest również bardzo ważnym gatunkiem ze względu na rolę ekologiczną, jaką pełni w ekosystemie – m.in. reguluje populacje mniejszych gatunków ryb.

Powszechnemu występowaniu szczupaka w polskich wodach śródlądowych towarzyszy drastyczne zmniejszanie się już od lat 70 liczebności populacji występujących w przybrzeżnych wodach morskich. Taka sytuacja jest wprawdzie charakterystyczna dla znacznej części Europy, jednakże w wodach polskich możemy mówić wręcz o stanie bliskim wyginięcia tego gatunku (Zatoka Pucka, Fig. 1). Powodem złego stanu zasobów szczupaka w wodach morskich jest w pewnym stopniu nieracjonalna gospodarka rybacka i kłusownictwo, ale przyczyną najistotniejszą są zmiany w strefie przybrzeżnej, która ma kluczowe znaczenie, jako obszar tarliskowy tego gatunku – szczupak w wodach morskich jest gatunkiem anadromicznym, wchodzącym na tarło do wody słodkiej (uwaga: w innych rejonach Bałtyku występuje również populacja rozradzająca się bezpośrednio w wodach słonawych).



Rys. 1. Płowy szczupaka w Zatoce Puckiej (Jackowski, 2002).

Jednym z możliwych wyjaśnień spadku liczebności szczupaka w Zatoce Puckiej jest więc, podobnie jak w innych rejonach bałtyckich, brak odpowiednich siedlisk tarlowych. Główną przyczyną zaniku naturalnych terenów tarliskowych jest natomiast

regulacja rzek skutkująca zanikiem rozlewisk oraz powstawanie konstrukcji hydrotechnicznych blokujących dostęp do lokalnych rzek i mniejszych kanałów. W obecnej sytuacji, istnienie populacji szczupaka w Zatoce Puckiej jest podtrzymywane poprzez zarybienia narybkiem pochodzącym z podchowu w ośrodkach hodowlanych, w oparciu o tarlaki „słodkowodne” (zarybienia Płutnicy przez PZW oraz okazyjne zarybienia w ramach projektów badawczych). Skutkiem jest praktycznie brak w wodach Zatoki Puckiej lokalnej populacji anadromicznej. Tak naprawdę jest to populacja szczupaka słodkowodnego.

Ten stan rzeczy wymusza wręcz konieczność lepszego poznania ekologii gatunku, zwłaszcza w okresie wczesnego rozwoju - procesy zachodzące w okresie rozwoju embrionalnego, larwalnego oraz juvenilnego mogą lokalnie skutkować nawet 100% śmiertelnością, przez co mają szczególne znaczenie dla stanu poszczególnych populacji ryb. Równie istotne jest zweryfikowanie dokładności oraz dopracowanie metod badawczych, na przykład zweryfikowanie zależności pomiędzy wzrostem somatycznym larw a tempem wzrostu ich otolitów.

### **Wpływ zasolenia na rozwój embrionalny szczupaka**

W sytuacji ograniczonego dostępu do wody słodkiej pojawia się pytanie, czy populacja szczupaka Zatoki Puckiej może się rozmnażać bezpośrednio w słonawych wodach zatoki. Czy istnieje poziom zasolenia, który pozwala na reprodukcję bezpośrednio w wodach zatoki przy ujściach rzek, gdzie zasolenie jest stosunkowo niskie? Istnieje wiele doniesień o udanym tarle szczupaka w wodach zasolonych, ale odnoszą się one do osiadłych populacji słonawych (Westin i Limburg, 2002; Lappalainen i in., 2008; Lehtonen i in., 2009; Engstedt i in., 2010; Jørgensen i in., 2009, 2010; Jacobsen i in., 2017; Möller i in., 2020), a taka populacja w Zatoce Puckiej nie występuje. Chociaż dostępne są informacje na temat zjawiska adaptacji populacji słodkowodnych do warunków zasolonych (Sunde i in., 2018), brak jest tego typu danych dla Zatoki Puckiej. Warto również zastanowić się, jak wpływa na wydajność tarła sytuacja, w której ikra złożona na słodkowodnych terenach podmokłych położonych bezpośrednio w strefach przybrzeżnych zostanie zalana wodą słonawą, tego rodzaju dane również nie są dostępne.



Rys. 2. Ikra zaoczkowana szczupaka oraz larwa tuż po wylęgu.

Powyższych zagadnień dotyczy [Publikacja nr 1](#), bazująca na materiale (ikra) pochodzącym od tarlaków słodkowodnych - obecna populacja szczupaka Zatoki Puckiej wywodzi się głównie z zarybień z populacji słodkowodnej. Pierwotna populacja anadromiczna, która podejmowała migracje tarłowe do rzek, tak naprawdę już nie istnieje. W niniejszej pracy rozważano: 1) możliwość zapłodnienia i rozwoju ikry szczupaka w wodach słonawych (zapłodnienie i rozwój w 0, 4 i 7 PSU) oraz 2) jakie mogą być skutki zalania tarlisk (naturalnych lub odtworzonych) położonych na rozlewiskach lub w kanałach rzek w strefie przybrzeżnej (przeniesienie ikry zapłodnionej w 0 PSU do 4 i 7 PSU). Zastosowany w doświadczeniach zakres zasolenia wód odzwierciedlał zasolenie wód słodkich (grupa kontrolna), wód przejściowych w dolnym biegu rzek wpadających do Bałtyku oraz wód przybrzeżnych Zatoki Puckiej. W pracy opisano również aktualny stan populacji szczupaka w Zatoce Puckiej, przedstawiono dane o połowach szczupaków w latach 1964-2019, omówiono przyczyny drastycznego spadku liczebności tej populacji oraz możliwe strategie zarządzania.

### **Wpływ temperatury wody na tempo wzrostu larw szczupaka**

Kolejnym bardzo istotnym zagadnieniem w odniesieniu do rozrodu szczupaka w rejonie Zatoki Puckiej jest wpływ temperatury na wczesne stadia rozwojowe. Tarło szczupaka na obszarze Zatoki Puckiej odbywa się wczesną wiosną w okresie od marca do maja w wodach o temperaturze od 5°C do 12°C, a siedlisko tarłowe szczupaka obejmuje płytkie

obszary zalewowe bogate w roślinność oraz inne złożone struktury na dnie. Oczywiście, tak, jak zostało to opisane w poprzednim podrozdziale, problemem jest obecnie dostęp do takich tarlisk. Ponieważ głębokość wody na terenach zalewowych zazwyczaj jest niewielka (często ok. 30-50 cm), temperatura wody, nawet wczesną wiosną, w słoneczne dni może w okresie pierwszych tygodni życia larw przekraczać 20°C. Dlatego tak istotne jest sprawdzenie, jaki wpływ na tempo wzrostu larw ma temperatura w szerokim zakresie jej wartości. Należy podkreślić, że tempo wzrostu w okresie larwalnym i juwenilnym ryb może być czynnikiem w znacznym stopniu decydującym o sukcesie rozrodczym. Jedną z ważniejszych hipotez rekrutacyjnych (growth-mortality hypothesis) zakłada, że lepszą przeżywalnością charakteryzują się osobniki większe i szybciej rosnące (Anderson, 1988; Miller i in., 1988). Znajomość zakresu tempa wzrostu larw oraz tego, w jakim stopniu jest ono uzależnione od warunków środowiskowych (np. temperatury), jest więc kluczowym zagadnieniem w badaniach ekologii ryb. Należy tu brać również pod uwagę możliwość wystąpienia temperatur przekraczających optimum dla wzrostu, a często wręcz redukujących tempo wzrostu.

Wpływ temperatury wody (10, 15 oraz 22°C) na tempo wzrostu larw i wczesnego narybku szczupaka w pierwszym miesiącu życia, czyli w okresie kiedy stadia młodociane przebywają na słodkowodnych terenach tarliskowych, określono w [Publikacji nr 2](#).

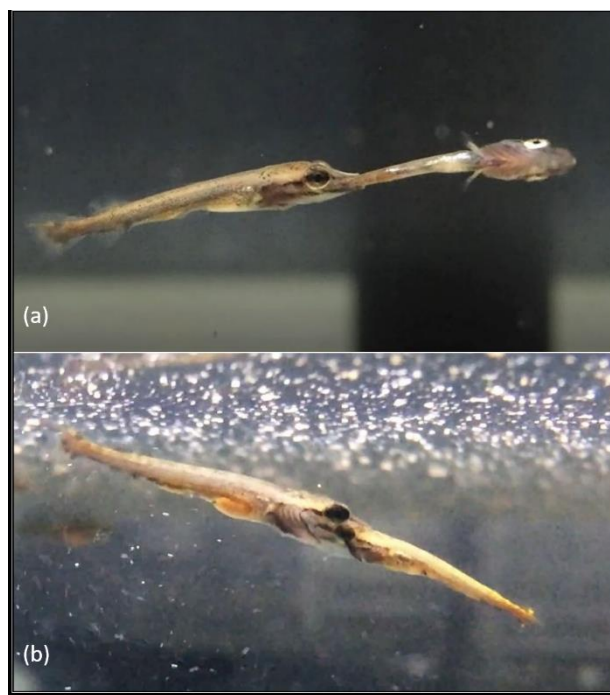


Rys. 3. Narybek letni szczupaka po zarybieniu w strefie przybrzeżnej Zatoki Puckiej.

## **Wpływ temperatury wody na kanibalizm w okresie larwalnym oraz wczesno-juwenilnym szczupaka**

W środowisku naturalnym kanibalizm jest jednym z mechanizmów regulujących liczebność tego gatunku, co ma istotne implikacje dla rekrutacji poszczególnych roczników szczupaka (Craig i Kipling, 1983), a także dla struktury danych populacji (Skov i Nilsson, 2018). Jest to szczególnie ważne w przypadku populacji nadmiernie eksploatowanych, które można spotkać na przykład w wielu obszarach przybrzeżnych Morza Bałtyckiego (Nilsson, Engstedt i Larsson, 2014; Larsson i in., 2015; Skov i Nilsson, 2018). Co istotne, kanibalizm, jako sposób odżywiania pojawia się już u larw i małego narybku. Kanibalizm występujący we wczesnym okresie życia szczupaka może odgrywać istotną rolę nie tylko w środowisku naturalnym, ale również w warunkach akwakultury, gdyż może powodować straty całkowite przekraczające 90% (Szczepkowski, 2009). Znaczenie i potencjalne konsekwencje występowania kanibalizmu pośród stadiów larwalnych i młodocianych sprawiają, że zrozumienie tego zjawiska jest niezmiernie istotne. Zdolność do zjadania rodzeństwa we wczesnym okresie życia, a więc jeszcze zanim nastąpi rozproszenie narybku do obszarów oddalonych od miejsca rozrodu, jest ułatwiona dzięki dużemu rozwarciu szczęk oraz dobrze rozwiniętym zębom już w momencie osiągnięcia wielkości około 12 mm, a więc w krótkim czasie po wylęgu (Giles i in., 1986, Pospisilova, 2019). Przy maksymalnym sukcesie wylęgu (w zależności od wielkości ciała, samica składa od 8 000 do 2 000 000 ziaren ikry, a zapłodnienie ikry w warunkach naturalnego tarła sięga nawet do 95%) początkowe zagęszczenie szczupaka w rejonach tarliskowych jest bardzo duże, co przy braku dostępności wystarczającej ilości innego pożywienia, może spowodować intensywny kanibalizm i znaczne zmniejszenie ilości potomstwa w danym rejonie. Na intensywność zjawiska kanibalizmu wczesnych stadiów rozwojowych może również mieć wpływ wysoka temperatura w rejonie tarłowym – płytkie, nagrzane rozlewiska.





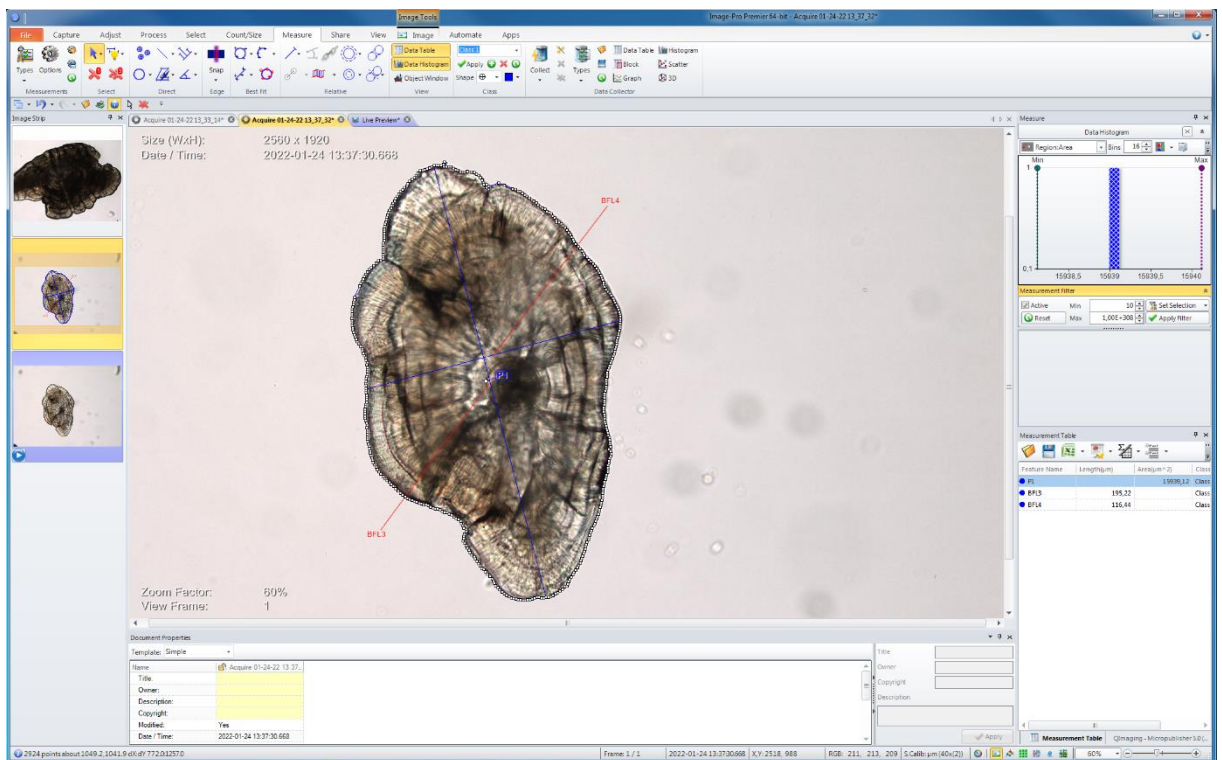
Rys. 4. Zdjęcie kanibalistycznych par szczupaków (*Esox lucius*) reprezentujących dwa rodzaje ataku: a) atak od strony ogona, b) atak od strony głowy.

W [publikacji nr 3](#) rozważaliśmy dla larw i narybku szczupaka w pierwszym miesiącu życia (9-27 mm SL), jaki wpływ ma temperatura wody (10, 15, 22°C) na wiek i wielkość drapieżników podczas pierwszego ataku kanibalistycznego, na intensywność tego zjawiska oraz na stosunek wielkości ofiary do drapieżnika.

### **Fluktuacyjna asymetria otolitów jako bioindykator**

W celu określenia kondycji osobniczej ryb można zastosować szereg metod (np. współczynnik Fultona, analizy morfologiczne i histologiczne, analiza zawartości lipidów i białek, stosunek RNA do DNA, analiza szerokości przyrostów na otolitach). Wiele z tych metod jest jednak pracochłonna, kosztowna i wymaga zastosowania specjalistycznych urządzeń, co ogranicza możliwości ich zastosowania przy mniejszych próbkach. Alternatywnie, kondycja, czy też sprawność (ang.: fitness) organizmu, znajduje odzwierciedlenie w niestabilności rozwojowej organizmu (Zakharov, 1992). Niestabilność rozwojowa wyraża się natomiast w zmiennej asymetrii, tzw. (z ang.) „Fluctuating Asymmetry” (FA) (Palmer, 1994), która jest definiowana jako przypadkowe odchylenie od idealnego symetrycznego układu dwustronnego (Palmer,

1994). FA można obserwować u ryb w różnych organach, między innymi w wielkości lub kształcie otolitów (Diaz-Gil, 2015). Zakładając, że stres środowiskowy powoduje niestabilność rozwojową, duża asymetria wielkości otolitów może wskazywać na gorszy stan ryb, które doświadczyły niesprzyjającego środowiska. Związek między kondycją/fitnesem ryb a FA otolitów badano zarówno u ryb dorosłych, jak i larw (Escós i inni, 1995; Somarakis i inni, 1997; Fey i Hare, 2008; Fey i inni, 2019). Wyniki uzyskane w poszczególnych badaniach były jednak niespójne – w różnych sytuacjach FA okazywała się skutecznym lub nieskutecznym wskaźnikiem kondycji, czy też tempa wzrostu ryb.



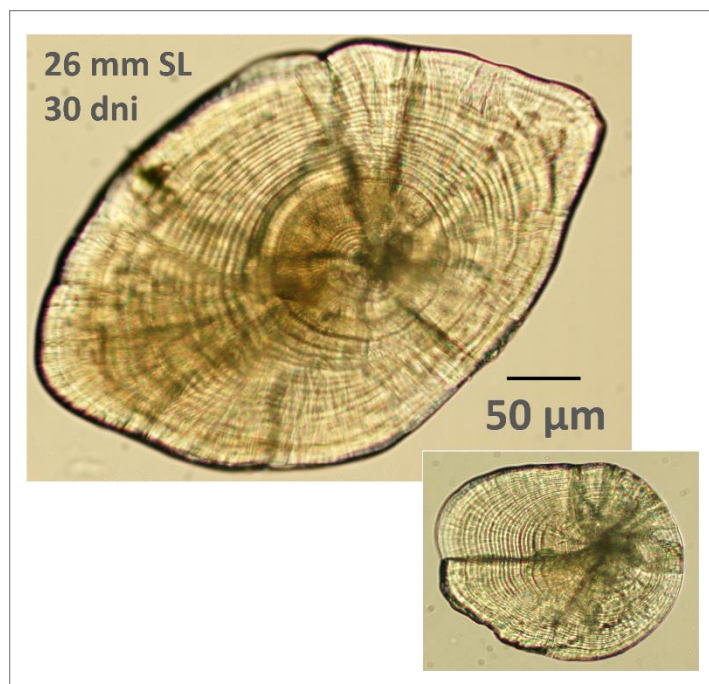
Rys. 5. Pomiary obwodu, długości oraz szerokości otolithu (strzałki) pod mikroskopem przy pomocy systemu analizy obrazu.

Celem jednego z zadań (Publikacja nr 3) było zweryfikowanie, czy larwy szczupaka należące do grupy kanibali i ofiar, wykazujące znaczące różnice w tempie wzrostu i kondycji, wykazują w pierwszym miesiącu życia również różnice w asymetrii wielkości otolitów (analizowano powierzchnię strzałek).

### **Zależność pomiędzy tempem wzrostu larw a tempem wzrostu otolitów**

Jednym ze źródeł informacji na temat wczesnej historii życia ryb są otolity – struktury zbudowane w dużej mierze z węglanu wapnia, wykorzystywane przez ryby jako część narządu równowagi, słuchu i orientacji (przegląd na temat powstawania otolitów i odkładania dobowych przyrostów – patrz Morales-Nin, 1992, 2000). Analiza mikrostruktury oraz zmian wielkości otolitów z wiekiem dostarcza liczne dane, dotyczące m.in. wieku, wzrostu, kondycji, dat wylęgu, obszaru geograficznego pochodzenia oraz śmiertelności na poziomie osobniczym oraz populacji larw (Campana i Neilson, 1985; Francis, 1990; Secor i Dean, 1992; Campana i Jones, 1992; Campana, 2005; Berg i in., 2017), osobników kilkumiesięcznych (Fey i Linkowski, 2006), a nawet dorosłych (Hussy i in., 2010). Występowanie ścisłego związku pomiędzy tempem wzrostu ryb a tempem wzrostu ich otolitów, umożliwia na przykład wykonywanie wstecznych odczytów tempa wzrostu. Ścisłość tej zależności nie została jednakże do tej pory zweryfikowana dla szczupaka. Zdarza się, że wzrost otolitów jest regulowany w większym stopniu przez temperaturę niż przez wzrost somatyczny danego osobnika (Fey, 2001). Dodatkowo, szybciej rosnące osobniki mogą mieć względnie mniejsze otolity. Taki stan rzeczy może znacząco wpływać na przykład na niedokładności uzyskiwanych krzywych wzrostu (Mosegard, 1988, Fey, 2001). Brak jest jednak danych umożliwiających zweryfikowanie występowania tego zjawiska dla wczesnych stadiów rozwojowych szczupaka.





Rys. 6. Mikrostruktura otolitów szczupaka – strzałka i kamyczek.

Przeprowadzone prace eksperymentalne (Publikacja nr 2) pozwoliły na uzyskanie do analiz osobników rosnących w szerokim zakresie temperatur (10, 15 oraz 22°C), a przez to charakteryzujących się bardzo zróżnicowanym tempem wzrostu. Zweryfikowano, na ile tempo wzrostu otolitów (a więc również szerokość przyrostów na otolitach) odzwierciedla wzrost somatyczny larw. Określono również, na ile temperatura wpływa na zależność wielkość ryby -wielkość otolitu.

## **Cel badań, hipotezy oraz zadania badawcze**

Celem badań było pogłębienie wiedzy z zakresu ekologii wczesnych stadiów rozwojowych szczupaka (*Esox lucius*), poprzez weryfikację, w oparciu o prace eksperymentalne, pięciu hipotez badawczych.

**Hipoteza I:** Zasolenie nie wpływa na rozwój embrionalny szczupaka

Zadania badawcze:

- 1) Określenie wpływu zasolenia 0, 4 i 7 PSU na prawidłowość rozwoju embrionalnego szczupaka.
- 2) Określenie wpływu zasolenia 0, 4 i 7 PSU na sukces wylęgu szczupaka.

**Hipoteza II:** Temperatura nie wpływa na tempo wzrostu larw szczupaka

Zadania badawcze:

- 1) Określenie wpływu temperatury (10, 15, 22°C) na tempo wzrostu larw szczupaka w pierwszych 30 dniach życia.

**Hipoteza III:** Temperatura nie wpływa na intensywność kanibalizmu stadiów młodocianych szczupaka

Zadania badawcze:

- 1) Określenie wpływu temperatury (10, 15, 22°C) na czas rozpoczęcia zjawiska kanibalizmu u młodocianych stadiów szczupaka.
- 2) Określenie wpływu temperatury (10, 15, 22°C) na intensywność kanibalizmu u szczupaka w pierwszych 30 dniach życia.
- 3) Określenie wpływu temperatury (10, 15, 22°C) na stosunek wielkości ofiary do wielkości drapieżcy w pierwszych 30 dniach życia.

**Hipoteza IV:** Asymetria fluktuacyjna otolitów nie jest bioindykatorem kondycji i sprawności larw szczupaka

Zadania badawcze:

- 1) Określenie różnicy w asymetrii fluktuacyjnej wielkości otolitów (powierzchnia strzałki) pomiędzy kanibalami a ofiarami.

**Hipoteza V:** Otolity wczesnych stadiów rozwojowych szczupaka nie stanowią skutecznego narzędzia w badaniach ekologii tego gatunku

Zadania badawcze:

- 1) Określenie tempa wzrostu otolitów stadiów młodocianych szczupaka w pierwszych 30 dniach życia w odniesieniu do wzrostu somatycznego (w temperaturze 10, 15 i 22°C).
- 2) Określenie wpływu temperatury (10, 15, 22°C) na zależność wielkość ryby – wielkość otolitu u szczupaka w pierwszych 30 dniach życia.

## Material i Metody

Prace wykonywano w ramach dwóch eksperymentów dotyczących: 1) wpływu zasolenia na rozwój embrionalny szczupaka (eksperyment I) oraz 2) wpływu temperatury na wzrost larw, intensywność zjawiska kanibalizmu oraz wzrost otolitów stadiów młodocianych szczupaka (eksperyment II). System eksperymentalny składał się z trzech oddzielnych układów recyrkulacyjnych z zamkniętym obiegiem wody, umożliwiających wykonanie trzech powtórzeń badanego wariantu. Każdy z tych układów był wyposażony w akwaria o objętości 55L, filtr mechaniczno-biologiczny oraz filtr UV. System ten znajdował się na terenie Akwarium Gdynskiego.

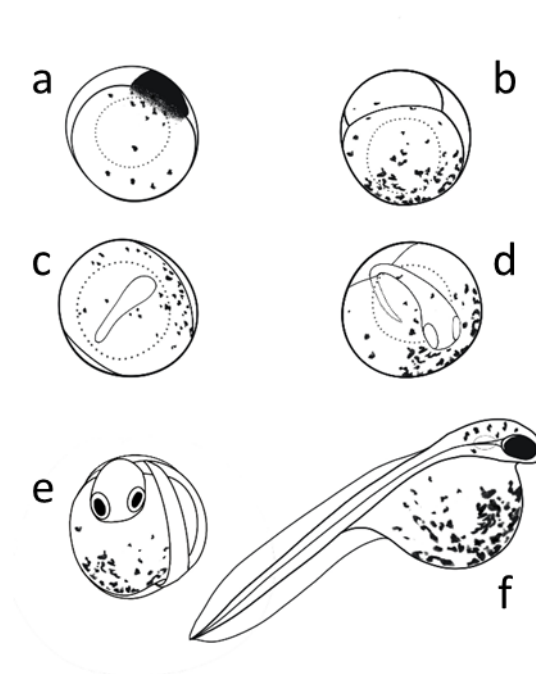


Rys. 7. System eksperymentalny, inkubacja ikry w różnych wariantach zasoleniowych.

### **Eksperyment I – Hipoteza I (Publikacja nr 1)**

Eksperyment został przeprowadzony w dwóch wariantach: 1) Ikra została zapłodniona w wodzie słodkiej, a następnie przeniesiona do słoik inkubacyjnych włączonych w obieg wody o różnym zasoleniu (~0, 4, 7 PSU); 2) Ikra została zapłodniona bezpośrednio w wodzie o różnym zasoleniu (~0, 4, 7 PSU), a następnie przeniesiona do słoik inkubacyjnych włączonych w obieg wody o zasoleniu takim samym, jak podczas zapłodnienia.

Inkubacja ikry trwała od 109 do 135 stopniodni, w zależności od wariantu zasolenia. Próbkę ikry (po 50 sztuk z każdego wariantu zasoleniowego) były pobierane codziennie w celu określenia poszczególnych etapów oraz prawidłowości rozwoju ikry przy pomocy systemu analizy obrazu (Nis Elements, Nikon) sprzężonego z mikroskopem binokularowym Nikon SMZ-18 (Nikon Corp., Japan). Poszczególne stadia rozwoju określane były na podstawie sześciostopniowej skali: stadium powstanie blastuli, gastrulacja, tworzenie się zarodka, zarodek z miseczkami optycznymi, zarodek zaoczkowany oraz larwa tuż po wylęgu (Rys. 8). Powyższa skala została określona na podstawie opisów rozwoju jaj szczupaka przedstawionych przez Raata (1988) i Coopera (2016).



Rys. 8. Sześciostopniowa skala rozwoju poszczególnych stadiów ikry szczupaka: stadium powstanie blastuli (a), gastrulacja (b), tworzenie się zarodka (c), zarodek z miseczkami optycznymi (d), zarodek zaoczkowany (e) oraz larwa tuż po wylęgu (f).

W sumie, określono stadium rozwojowe dla 3500 ziaren ikry. Określono również procentowy wskaźnik sukcesu wylęgu na podstawie liczby wyklutych larw w odniesieniu do liczby poddanych zapłodnieniu jaj. Analizę danych przeprowadzono przy użyciu odpowiednich metod statystycznych w zależności od porównywanego parametru.

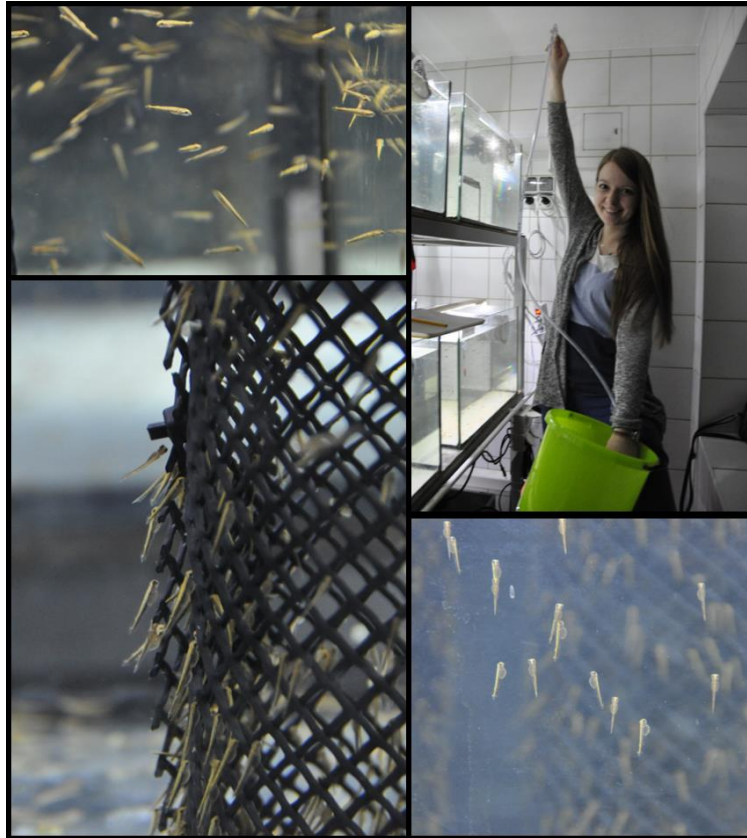
## **Eksperyment II – Hipoteza II i V (Publikacja nr 2), Hipoteza III i IV (Publikacja nr 3)**

Wylęg szczupaka umieszczono w akwariach w wodzie słodkiej o początkowej temperaturze 10°C. Następnie, w ciągu 2 dni, stopniowo zwiększano temperaturę wody w poszczególnych wariantach eksperymentalnych do 15 oraz 22°C. Próbkę larw do analiz tempa wzrostu somatycznego oraz tempa wzrostu otolitów (po 30 osobników z każdego wariantu) były pobierane co trzy dni od momentu rozpoczęcia eksperymentu, a następnie konserwowane w szklanych fiolkach w 96% alkoholu. Dodatkowo, sześć razy dziennie monitorowano w każdym akwarium przez 15 minut obecność par kanibalistycznych, czyli kanibala pływającego ze swoją zdobyczą w pysku. Takie pary były pobierane i konserwowane w 96% alkoholu do dalszych analiz.

W sumie analizie poddano 810 osobników (badania tempa wzrostu somatycznego i tempa wzrostu otolitów) oraz 71 par kanibalistycznych. Każdy osobnik został zmierzony (długość standardowa) przy pomocy elektronicznej suwmiarki z dokładnością do 0,1 mm. Długość ta została skorygowana w związku z kurczliwością larw w płynie konserwującym, z wykorzystaniem odpowiedniego współczynnika korygującego (Greszkiewicz i Fey, 2018). Następnie, ze wszystkich larw, również w parach kanibalistycznych, wyekstrahowano otolity - lewą i prawą strzałkę oraz lewy i prawy kamyczek.

Wielkość otolitów mierzono pod mikroskopem w świetle przechodzącym (Nikon Eclipse 80i) za pomocą systemu analizy obrazu (Image-Pro Premier). W sumie zmierzono 1440 otolitów (długość i szerokość) do analizy ich tempa wzrostu oraz 284 otolity (powierzchnia, długość i szerokość) do analizy fluktuacyjnej asymetrii otolitów pobranych od par kanibalistycznych.





Rys. 9. Hodowla wczesnych stadiów rozwojowych szczupaka w systemie eksperymentalnym.

**Artykuł I** - Greszkiewicz Martyna, Fey Dariusz P., Lejk Adam M., Zimak Michał (2022). The effect of salinity on the development of freshwater pike (*Esox lucius* L.) eggs in the context of drastic pike population decline in Puck Bay, Baltic Sea. *Hydrobiologia*  
<https://doi.org/10.1007/s10750-022-04893-x>





# The effect of salinity on the development of freshwater pike (*Esox lucius*) eggs in the context of drastic pike population decline in Puck Lagoon, Baltic Sea

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**Abstract** Over the past few decades, the number of available spawning grounds for anadromous pikes, *Esox lucius*, on the Baltic Sea coast has been significantly reduced resulting in a drastic pike population decline in those areas. It is therefore a question if saline Baltic waters may support the spawning of anadromous pike. In the present experimental study, salinity had a harmful impact on the development of eggs from pikes of freshwater origin. The hatching success rates were 0% at 7PSU and only 16.7% at 4PSU, while hatching success at 0PSU was 89.7%. However, when the eggs were fertilized in fresh water and transferred to the tested salinities after 1 h, salinity had a positive effect on egg development and hatching success (0PSU: 80%; 4PSU: 83.3%; and 7PSU: 93.7%). We concluded that the spawning of

the freshwater pike population in the saline water of the Baltic Sea is most likely not successful. Simultaneously, inflows of coastal marine waters into near-shore spawning grounds do not have a harmful effect on egg development if the eggs are already fertilized. The main management measures that can be suggested are to rebuild the anadromous population by spawning ground reconstruction. Sea-spawning population introduction can also be considered.

**Keywords** Egg incubation · Pike fry · Brackish waters · Reproduction success · Fish restoration

## Introduction

Pike (*Esox lucius* Linnaeus, 1758) is an economically valuable species (Pierce et al., 1995), and as a top predator, it also plays an important role in ecosystems by regulating fish population abundance (Craig, 1996, 2008; Eriksson et al., 2011; Crane et al., 2015; Persson et al., 2018). Pikes occur in both the fresh waters of lakes and rivers and in the coastal zones of brackish waters, for example, in the Baltic Sea (Ojaveer, 1981; Nilsson, 2006; Jacobsen et al., 2017; Möller et al., 2020). The pike spawning period is from February to May in waters with temperatures from 5 to 14°C (Raat, 1988; Horbowa & Fey, 2013). Anadromous Baltic pike populations spawn in fresh waters in seasonal wetlands (Müller, 1986; Nilsson, 2006; Engstedt et al., 2010; Nilsson et al., 2014), while

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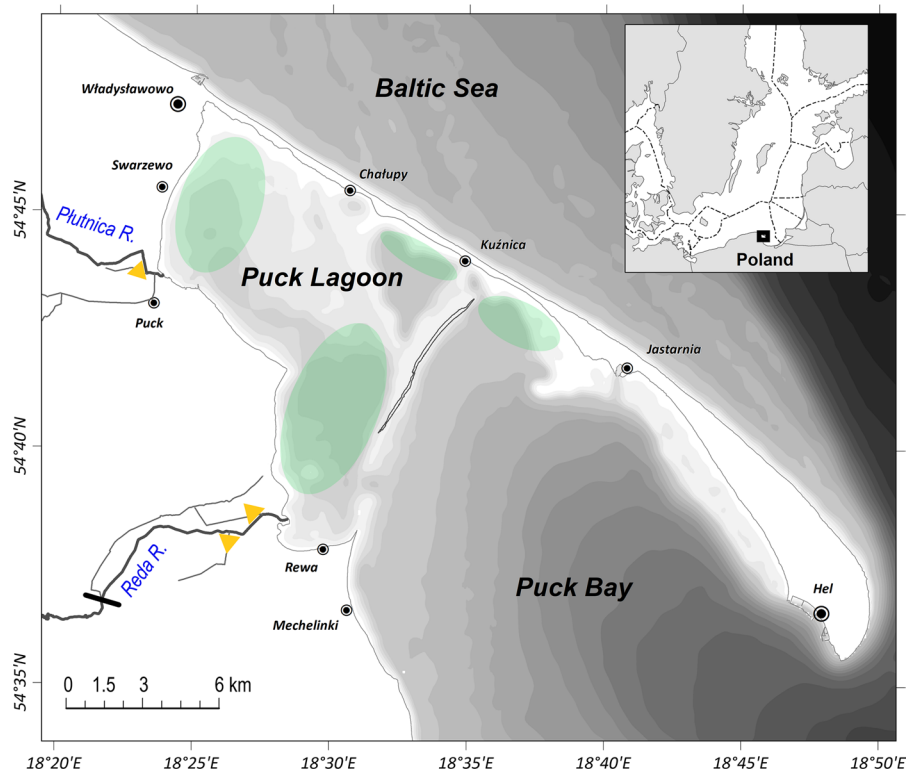
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resident brackish pike populations that do not require fresh water for successful reproduction spawn directly in shallow saline waters in coastal bays (Andersson et al., 2000; Westin & Limburg, 2002; Lappalainen et al., 2008; Lehtonen et al., 2009; Engstedt et al., 2010; Jørgensen et al., 2010; Rohtla et al., 2012; Jacobsen et al., 2017; Möller et al., 2019, 2020). After spawning, the two populations mix during the feeding season in coastal waters (Engstedt et al., 2014). Eggs are deposited on vegetation (Bry, 1996; Raat, 1988; Craig, 2008; Jacobsen & Engstrom-Öst, 2018) and develop, depending on water temperature, within eight to 21 days (Frost & Kipling, 1967; Jørgensen et al., 2010; Cooper, 2016; Fey et al., 2018, 2019). Exogenous feeding starts a few days before yolk absorption, for example, 9 days post-hatching at 12°C (Pospisilova et al., 2019). Immediately after yolk-sac absorption, which occurs at 12°C within 16–22 days post-hatching (Pospisilova et al., 2019), cannibalistic behavior starts (Greszkiewicz & Fey, 2020). The growth of larvae and early juveniles during the first weeks may be higher than 1 mm d<sup>-1</sup> if the temperature is above 20°C (Fey & Greszkiewicz, 2020). For a

comprehensive and recent review on pike biology and ecology, see Skov & Nilsson (2018).

Resident brackish pike populations occur in the coastal zones of Denmark (Jørgensen et al., 2010; Jacobsen et al., 2017), Finland (Lappalainen et al., 2008; Lehtonen et al., 2009), Sweden (Andersson et al., 2000; Nilsson, 2006; Engstedt et al., 2010), Germany (Möller et al., 2019), and Estonia (Rohtla et al., 2012). The proportion of brackish populations in different areas varies; for example, it can be as high as 94% in Rugen, Germany (Möller et al., 2019) and 56% on the southern Sweden coast (Engstedt et al., 2010) or as low as 18% in Estonia (Rohtla et al., 2012), while there is no information on the occurrence of resident brackish populations in the coastal waters of Poland. Maps from the 1920s indicate that there was a pike protection area in Puck Lagoon that could be an indication of pike spawning in the bay (Fig. 1). However, it could also be an anadromous population under protection. Resident brackish pike populations have no problems with access to spawning grounds located directly in bays, but other factors could reduce spawning success, such as increased eutrophication and turbidity (Sandström & Karås,

**Fig. 1** Map of Puck Lagoon and Puck Bay with the Plutnica and Reda Rivers marked, which no longer provided appropriate freshwater pike spawning habitats because of the drainage of surrounding lands and the location of hydrotechnical installations (triangles) constructed in the 1970s. The pike protection area designated in the 1920s was located in the saline waters of the bay (marked with green translucent area), which suggests that presence of a resident brackish pike population in this geographical area could not be excluded



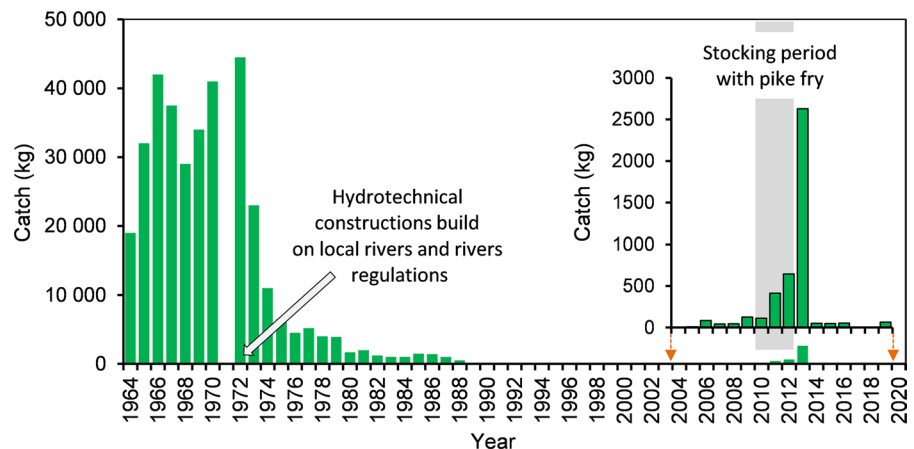
2002; Lehtonen et al., 2009; Olsson, 2019), changes in communities of fish that prey on pike eggs, such as stickleback (Nilsson, 2006; Donadi et al., 2020), or inflows of saltwater through the Danish Straits that can cause significant mortality in both early developmental stages and adults (Dahl, 1961; Jacobsen et al., 2007, 2008; Jørgensen et al., 2010). In contrast, the reproduction of anadromous pike populations inhabiting brackish waters is frequently limited as a result of human activities, including the construction of barriers that restrict access to the upper reaches of rivers and the wetlands located there (Casselmann & Lewis, 1996), as well as river regulation and land drainage that drastically decrease wetlands (Hoffman et al., 2000). As a consequence of these factors that affect both anadromous and resident brackish pike populations, many Baltic Sea populations are close to extinction, as indicated by pike catches of commercial and recreational fisheries (Andersson et al., 2000; Nilsson, 2006; Lehtonen et al., 2009; Nilsson et al., 2014; Larsson et al., 2015; Olsson, 2019).

Puck Lagoon is one example of a brackish bay with restricted access to freshwater spawning grounds in the Baltic Sea. Data from fisheries catches indicate that the pike population in this reservoir has declined drastically over the past few decades. From the 1960s to the mid-1970s, pike catches in Puck Lagoon and Puck Bay were 40 to 50 tons annually (Jackowski, 2002). Since this period, catches have decreased steadily, and since the 1990s, annual catches have been in the range of 50–100 kg (Jackowski, 2002) (Fig. 2). Catches from Puck Lagoon constitute approximately 95% of the total catches presented.

As is the case in other Baltic regions, one possible explanation for the drop in pike abundance in Puck Lagoon is the absence of appropriate spawning habitats resulting from vanishing wetlands from river regulation and hydrological constructions blocking access to local rivers, which is a current problem (see Fig. 1). Because of their considerably limited reproductive success, the continued existence of pike populations currently depends on stocking (data in this study), which is conducted in Puck Lagoon with fry obtained from freshwater spawners and is only performed during research projects such as ZOSTERA (2010–2013) and PIKE (2020–2023). Although the Polish Angling Association regularly stocks small feeding fry, this is only done in rivers that have very restricted connections with Puck Lagoon waters. Since freshwater fry are used for stocking, the pike population in Puck Lagoon is not a typical self-reproducing anadromous population but rather one of freshwater specimens.

In spawning grounds with restricted access to fresh water, the question arises of whether it is possible for freshwater populations to reproduce directly in brackish bay waters. Is there a salinity level that permits reproduction directly in bay waters at river mouths where salinity is relatively low? There are many reports of successful pike spawning in saline waters, but these refer to resident brackish populations (Westin & Limburg, 2002; Lappalainen et al., 2008; Lehtonen et al., 2009; Engstedt et al., 2010; Jørgensen et al., 2010; Jacobsen et al., 2017; Möller et al., 2020), and there is no such population in Puck Lagoon. Although information on the phenomenon of freshwater populations adapting to saline conditions

**Fig. 2** Pike catches in Puck Lagoon and Puck Bay in 1964–2004 (Jackowski, 2002) and 2005–2019 (data from the Fisheries Monitoring Center in Gdynia, Poland). The gray area indicates 2010–2013 when pike fry stocking was performed, which corresponded to notable increases in catches



is available (Sunde et al., 2018), no data of this kind are available for Puck Lagoon. It is also worth considering how spawning efficiency is affected if eggs deposited in freshwater wetlands located directly in coastal zones are flooded with brackish saline waters; however, no data of this kind are available.

The aim of this study was to determine under experimental conditions the impact of water salinity (0, 4, and 7 PSU) on the success of egg development and larval hatching of pikes from a freshwater population. The current Puck Lagoon freshwater pike population originates from stocking with fishes from a freshwater population, while the original brackish population that undertook spawning migrations into rivers no longer exists. The option of fertilizing eggs in freshwater and transferring them to higher salinities of 4 and 7 PSU was considered in light of possible flooding in spawning grounds located in wetlands in coastal saline waters (natural or restored). The water salinity range used in the experiments reflected that of fresh water (control group), transitional waters in the lower reaches of rivers flowing into the Baltic Sea, and the waters of the coastal Puck Lagoon. This work also describes the current state of the pike population in Puck Lagoon, includes data on pike catches from 1964 to 2019, and discusses possible management strategies.

## Materials and methods

### Study area

Although the present study is based on data obtained under experimental conditions, the concept of the experiment is strongly linked to the drastic decline in the pike population in Puck Lagoon, Baltic Sea (Fig. 1), which has been noted since the 1970s (Fig. 2). Even if these data are not catch per unit effort (CPUE) data, they do indicate a decline. According to the Polish Fisheries Monitoring Center, which has collected electronic catch data since 2004, pikes are caught with the use of fyke nets and gill nets in Puck Lagoon. Because the same type of nets were the main fishing tools in 60s–80s catches (National Marine Fisheries Research Institute, unpublished data), the catch data presented in Fig. 2 for different years should be considered comparable. Figure 1 presents a map of the local *Plutnica* and *Reda* Rivers that

were regulated in the 1970s and are currently more akin to channels with limited vegetation than to natural rivers. Pump stations in their lower segments contributed to the disappearance of wetlands along these rivers and restricted access to shallower streams that branch off and flow away from the main river channels. The pike protection area designated in the 1920s is also noted on the map (redrawn from historical map from year 1920), which could suggest that a resident brackish pike population lived in Puck Lagoon at this time.

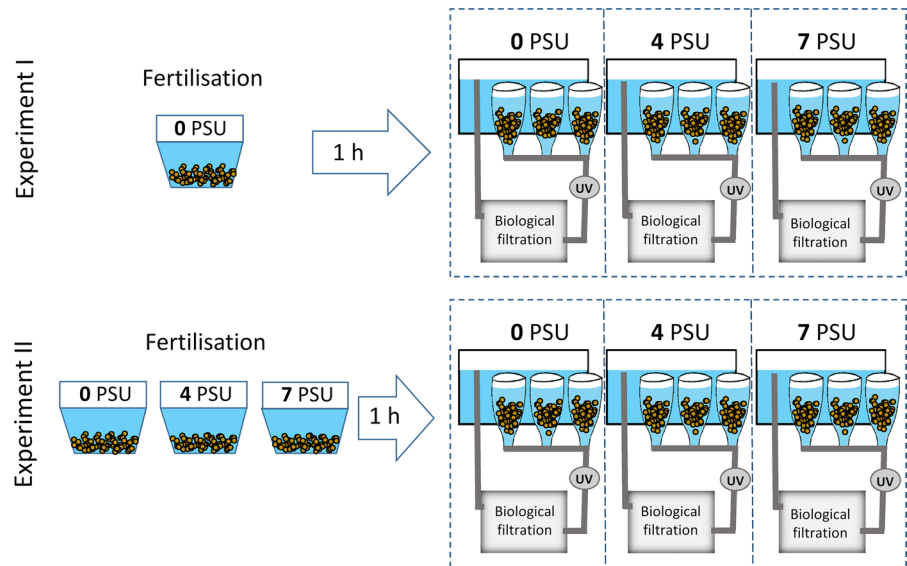
### Experimental system set-up

The experimental system set-up comprised three separate recirculating systems (Fig. 3). Each of them included three experimental mini Weiss jars of 0.5 l each connected to glass tanks that served as hatched larva containers. Each system included a 70 l PVC filtration-sedimentation tank that was filled with 20 l of biological media (Bioceramax Pro 600, 600 m<sup>2</sup> l<sup>-1</sup>, Aqual). A UV lamp was installed at the water flow. Each recirculating system permitted conducting the experiment at three different salinities (0, 4, and 7 PSU) and in three replicates for each salinity.

### Experimental conditions

Two experiments were conducted to study the effect of salinity on pike egg development. Eggs for the studies were obtained from four females (66–73 cm TL), and milt was obtained from two males (52–59 cm TL) caught in mid-April with fyke nets from freshwater populations in *Ostrzyckie Lake* (geographical position: 54° 15' 13.1" N; 18° 06' 15.8" E). The pikes were stripped directly after they were caught, and eggs were mixed with milt. Approximately 4.5 ml of milt was used. Fertilization (adding water) was performed after the eggs were transported to the laboratory. The transport took one hour. In the laboratory, the eggs were divided into two groups. In Experiment I, the eggs from one group were fertilized in fresh water (nonchlorine tap water). After one hour, the eggs were transferred to incubation jars with different salinities (salinity during the incubation time, mean ± SD): 0.18 ± 0.04; 4.0 ± 0.21; and 6.9 ± 0.17 PSU (Fig. 3). In Experiment II, the eggs from the other group were fertilized directly at different salinities and then transferred to incubation

**Fig. 3** Diagram of the experimental setup: Experiment I—eggs fertilized in fresh water at 0 PSU and transferred after 1 h to salinities of 4 and 7 PSU; Experiment II—eggs fertilized and incubated in salinities of 0, 4, and 7 PSU



jars (salinity during the incubation time, mean  $\pm$  SD:  $0.17 \pm 0.05$ ;  $4.1 \pm 0.26$ ; and  $7.0 \pm 0.15$  PSU) (Fig. 3). Water pumped from Puck Bay (7 PSU) was used to obtain different salinities. The quantity of eggs placed in each incubation jar was 200 ml in both experiments, which corresponds, as estimated by volumetric method, to ca. 10 000 eggs. Although some variation in the salinities in the three groups was noted, we referred throughout the publication to salinity Groups 0, 4, and 7 PSU. The light regime for simulating natural conditions in spring was set to 12 h light/12 h dark. The light period was from 08:00 to 20:00 and was regulated automatically. The temperature was set to  $11 \pm 0.4^\circ\text{C}$  (mean  $\pm$  SD) and measured twice daily at the same time as salinity. This temperature was within the optimum for pike reproduction ( $8\text{--}15^\circ\text{C}$ , Raat, 1988) that occurs during spawning periods in shallow, sun-heated coastal waters ( $8.9\text{--}13.8^\circ\text{C}$ , Nilsson, 2006). During the period from fertilization to larval hatching, egg samples (approximately 10 ml) were collected from each incubation jar daily to assess egg development.

This experimental set-up based on using pikes from a freshwater environment was intentional since pikes inhabiting Puck Lagoon originate from stocking with freshwater fry, and self-reproducing anadromous populations of a number of generations are absent from this area even if it cannot be excluded that occasionally some specimens come from natural spawning.

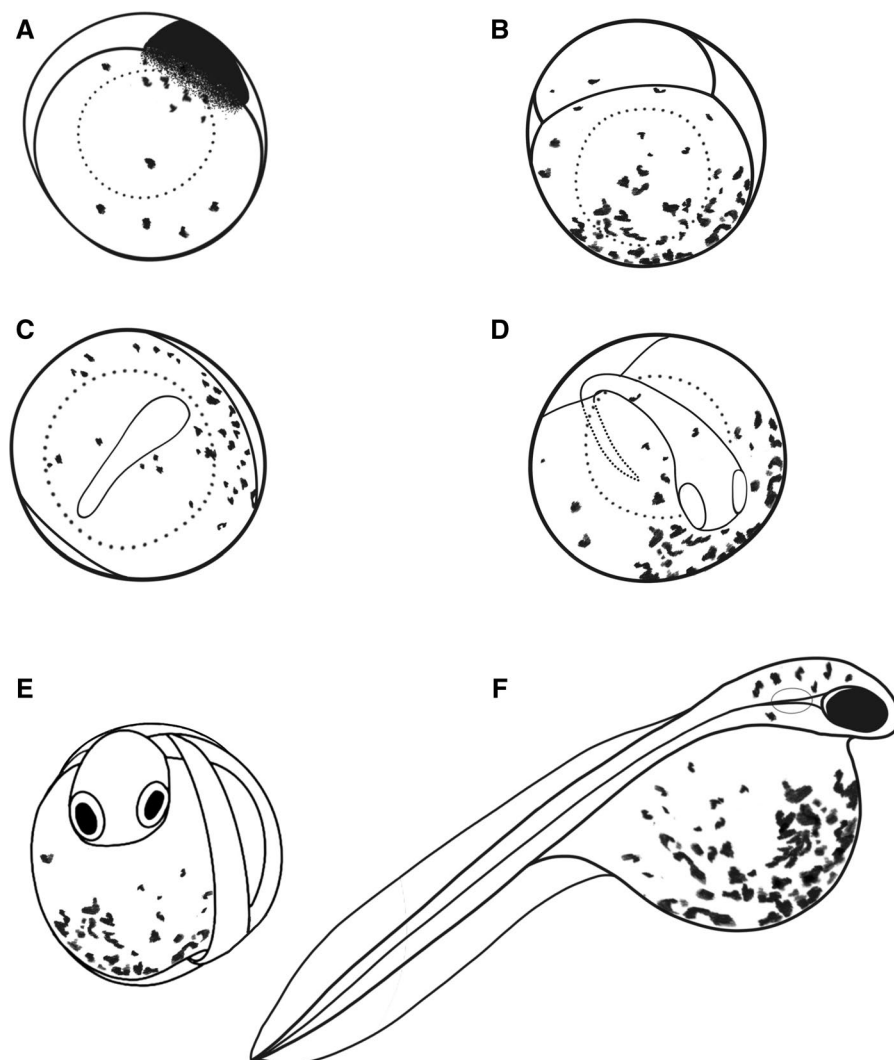
#### Analysis of egg development

The eggs collected daily from individual jars were mixed among the three replicates from each of the three salinities. Then, 50 eggs were chosen at random daily from each of the three salinity variants. The eggs were analyzed with an image analysis system (NIS Elements, Nikon) coupled with a Nikon SMZ-18 binocular microscope (Nikon Corp., Japan). The analyses included (1) determining the developmental stage of individual eggs according to the six-stage scale in Fig. 4 (blastula formation, gastrulation, embryo formation, embryos with optic cups, eyed embryos, and hatched larvae), which was based on descriptions of pike egg development by Raat (1988) and Cooper (2016), and (2) determining the hatching rate. The fertilization rate was not determined. Egg swelling, which is an indicator of successful fertilization, could not be detected retrospectively.

#### Data analysis

Hatching success (%) was estimated by counting the unhatched eggs and subtracting them from the number of fertilized eggs. Differences in hatching success among salinities in the experiments were evaluated with Kruskal–Wallis test for independent samples and the post hoc Dunn's test with Bonferroni correction for multiple tests. The statistical significance of the effect of salinity (0, 4, and 7 PSU)

**Fig. 4** Illustration of subsequent developmental stages of northern pike eggs: **A** stage 1—blastula formation; **B** stage 2—gastrulation; **C** stage 3—embryo formation; **D** stage 4—embryo with optic cups; **E** stage 5—eyed embryo; **F** stage 6—hatched larva



on egg development was evaluated separately for the two experiments with GLM repeated measure model (from the mixed-effects models category) with egg development stage as the categorical response variable with 6 levels, salinity as the between-subject variable (fixed effects factor), and degree-days as the within-subject variable (a random effects factor) with 13 (experiment I) and 10 (Experiment II) levels. The degree-days as a continuous variable has been added to the model as a random slope. In addition to the main effects, the interaction of between-subjects term with within-subjects term is included in the model as well. The assumption of the sphericity of the covariance matrix was verified with Mauchly's test. If this assumption was violated, a Greenhouse–Geisser

correction adjustment to the degrees of freedom was applied for the within-subject analysis. Model evaluation showed that the model residuals were normally distributed. Pairwise comparisons of salinity effects were evaluated with the post hoc Tukey HSD test. Pairwise comparisons among salinity groups within degree-days were based on estimated marginal means with a Bonferroni adjustment for repeated comparisons. Additionally, the rate of egg development was evaluated among the salinities by comparing the slopes of the line regressions fitted to the relationships between egg development stage and degree-days. The egg developmental stage is the same categorical response variable with 6 levels as in the GLM repeated measure analysis. The pairwise differences



between slopes for salinity groups were assessed with Tukey's HSD post hoc test. Statistica ver. 12.0 (TIBCO Software, Inc., Palo Alto, CA) and IBM SPSS Statistics ver. 26 (IBM Corp.) were used for the data analysis. Differences were considered statistically significant at  $P < 0.05$  ( $\alpha = 0.05$ ).

## Results

### Experiment I

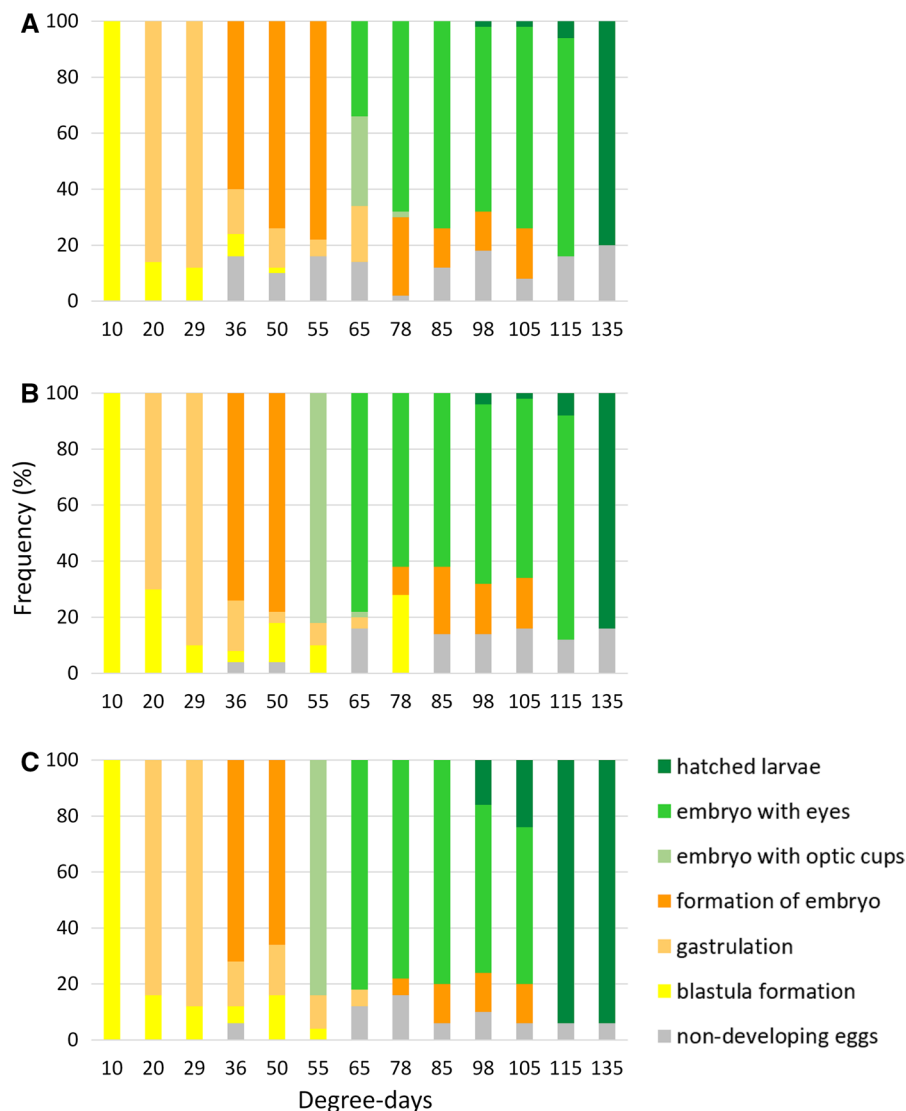
During Experiment I (eggs fertilized in fresh water and transferred after 1 h to incubate at 0, 4, and 7 PSU), all the eggs in the subsamples analyzed from the three salinities were fertilized. The effect of the degree-day (DD, temperature x day) on the egg

developmental stage was statistically significant (GLM repeated measure,  $P < 0.01$ ), but the effect of salinity was not (GLM repeated measure,  $P = 0.138$ ) (Table 1). However, the statistically significant interaction term between degree-days and salinity indicated that the magnitude of the within-subject differences was salinity dependent. Until 85 DD, egg development in the three salinity variants was very similar (Fig. 5 and S1), and no significant differences were observed, except for 55 DD (GLM repeated measure, salinity comparisons within DD based on estimated marginal means) (Table 2). The acceleration of egg development at 7 PSU from 98 DD resulted in significant differences at 115 DD when all the larvae hatched at 7 PSU. At 0 and 4 PSU, most of the eggs were still in the eyed embryo stage at 115 DD, and mass hatching occurred at 135 DD.

**Table 1** Mean SD developmental stage of pike eggs on given degree-days (DD) during incubation at salinities of 0, 4, and 7 PSU

	DD	Egg developmental stage						Homogeneous <sup>a</sup> groups	P value <sup>b</sup>
		0 PSU		4 PSU		7 PSU			
		Mean	SD	Mean	SD	Mean	SD		
Experiment I									
	10	1.00	0.00	1.00	0.00	1.00	0.00	0–7	NS
	20	1.86	0.05	1.70	0.05	1.84	0.05	0–7	NS
	29	1.88	0.04	1.90	0.04	1.88	0.04	0–7	NS
	36	2.21	0.12	2.62	0.12	2.54	0.12	0–7	NS
Experiment I—egg	50	2.53	0.12	2.56	0.12	2.50	0.12	0–7	NS
fertilization in fresh water	55	2.47	0.13	3.54	0.13	3.64	0.13	0; 4–7	$P < 0.05$
and transfer after 1 h to	65	3.39	0.24	4.07	0.24	4.23	0.24	0–7	NS
experimental salinities;	78	4.32	0.22	3.68	0.22	4.09	0.22	0–7	NS
Experiment II—egg	85	4.13	0.22	3.83	0.22	4.42	0.22	0–7	NS
fertilization directly at a	98	3.85	0.25	3.99	0.25	4.39	0.25	0–7	NS
given salinity. The stages	105	4.26	0.22	3.87	0.22	4.66	0.22	0–7	NS
were as follows: 1—blastula	115	4.27	0.23	4.49	0.23	5.64	0.23	0–4; 7	$P < 0.05$
formation, 2—gastrulation,	135	4.82	0.28	5.05	0.28	5.64	0.28	0–7	NS
3—embryo formation,	Experiment II								
4—embryos with optic	17	0.89	0.05	0.62	0.05	0.10	0.00	0; 4; 7	$P < 0.001$
cups, 5—eyed embryos, and	24	2.00	0.05	1.81	0.05	0.10	0.00	0; 4; 7	$P < 0.001$
6—hatched larvae. General	38	1.73	0.07	1.92	0.07	0.10	0.00	0–4; 7	$P < 0.001$
differences among salinities	47	1.69	0.10	1.79	0.10	0.10	0.00	0–4; 7	$P < 0.001$
and pairwise comparisons	54	2.98	0.18	2.18	0.18	0.10	0.00	0; 4; 7	$P < 0.001$
between salinities on a	70	4.21	0.20	2.22	0.20	0.10	0.00	0; 4; 7	$P < 0.001$
given DD were evaluated	77	4.41	0.19	1.89	0.19	0.10	0.00	0; 4; 7	$P < 0.001$
with GLM repeated	85	3.43	0.28	1.91	0.28	0.10	0.00	0; 4; 7	$P < 0.001$
measure model	93	4.39	0.35	1.72	0.31	0.10	0.00	0; 4; 7	$P < 0.001$
<sup>a</sup> Bonferroni adjustment for	109	6.00	0.28	3.75	0.28	0.10	0.00	0; 4; 7	$P < 0.001$
repeated comparisons was									
included									
<sup>b</sup> Comparisons within DD									
based on estimated mar-									
ginal means									

**Fig. 5** Comparison of pike egg development in Experiment I (fertilization in fresh water) on given sampling days (DD) at salinities of 0 (A), 4 (B), and 7 (C). Fifty eggs were analyzed from each of 13 samplings



The overall rate of egg development (i.e., slopes of linear regression lines fitted to the egg development stage at 0 DD data; Fig. 6) was significantly different among salinities (ANCOVA,  $P < 0.001$ ) (Table 2); however, the differences were only significant between 0 and 7 PSU and between 4 and 7 PSU (post hoc Tukey HSD,  $P < 0.001$ ). There were no differences between 0 and 4 PSU (post hoc Tukey HSD,  $P = 0.655$ ). The differences in slopes indicated faster egg development rates at 7 PSU than at 0 and 4 PSU.

Low egg mortality at all three salinities resulted in high rates of hatching success (80.0%—salinity 0 PSU; 83.3%—salinity 4 PSU; and 93.7%—salinity 7 PSU) (Table 3). The differences were statistically

significant overall (Kruskal–Wallis,  $P < 0.05$ ) and between 0 and 7 PSU (post hoc Dunn–Bonferroni test,  $P < 0.05$ ). The differences between 0 and 4 PSU and between 4 and 7 PSU were not statistically significant (post hoc Dunn–Bonferroni test,  $P = 0.89$  and  $P = 0.41$ ).

#### Experiment II

During Experiment II (eggs fertilized at 0, 4, and 7 PSU), at the beginning of the experiment, there were no signs of egg development in the eggs fertilized at 7 PSU (Figs. 7 and S2), while at 4 PSU, 16.7% of the eggs, and at 0 PSU, 5% of eggs, did not develop.



**Table 2** Results of GLM repeated measure model for the effect of salinity (between-subject variable) and degree-days (DD) (within-subject variable) on the pike egg developmental stage (categorical response variable) during the egg incubation period from fertilization to larval hatching

Experiment I—eggs fertilized in fresh water and transferred after 1 h to incubate in salinities of 0, 4, and 7 PSU; Experiment II—eggs fertilized in salinities of 0, 4, and 7

<sup>a</sup>Greenhouse–Geisser correction adjustment due to the violation of the sphericity assumption

	Type III SS	df	MS	<i>F</i>	<i>P</i>
Experiment I					
Between-subject differences					
Intercept	21,625.9	1	21,625.3	1381.9	<i>P</i> < 0.01
Salinity	62.9	2	31.5	2.0	<i>P</i> = 0.138
Error (Salinity)	2300.3	147	15.7		
Repeated measures <sup>a</sup>					
Degree-days	2960.8	2.6	1126.5	370.7	<i>P</i> < 0.001
Degree-days x Salinity	119.1	5.3	22.7	7.5	<i>P</i> < 0.001
Error (degree-days)	1173.4	386.4	3.0		
Experiment II					
Between-subject differences					
Intercept	4614.1	1	4614.1	1081.0	<i>P</i> < 0.001
Salinity	2406.2	2	1203.2	281.9	<i>P</i> < 0.001
Error (Salinity)	627.4	98	6.4		
Repeated measures <sup>a</sup>					
Degree-days	753.3	2.07	364.5	83.1	<i>P</i> < 0.001
Degree-days x Salinity	661.1	4.2	159.8	36.5	<i>P</i> < 0.001
Error (degree-days)	1332.6	303.9	4.4		

The effects of both salinity and degree-days on the differences in the egg developmental stage among the salinity groups (0, 4, and 7 PSU) were statistically significant (GLM repeated measure,  $P < 0.01$ ) (Table 1). Pairwise comparison showed significant differences among all the salinity pairs (0–4, 0–7, and 4–7 PSU) (post hoc Tukey HSD, all  $P < 0.05$ ). The interaction term between the effect of degree-days and salinity was also significant (GLM repeated measure,  $P < 0.01$ ), which indicated that the magnitude of within-subject differences was dependent on salinity. At 0 PSU, egg development proceeded normally, and after gastrulation, most of the eggs reached stage 5 (embryos with optic cups) at 54 DD, followed by the eyed embryo stage at 77 DD. At 4 PSU, egg development proceeded similarly to that at 0 PSU until 47 DD, but significant differences were noted from 54 DD (GLM repeated measure, salinity comparisons within DD based on estimated marginal means) (Table 1), and the development of most eggs at 4 PSU stopped at the gastrulation stage.

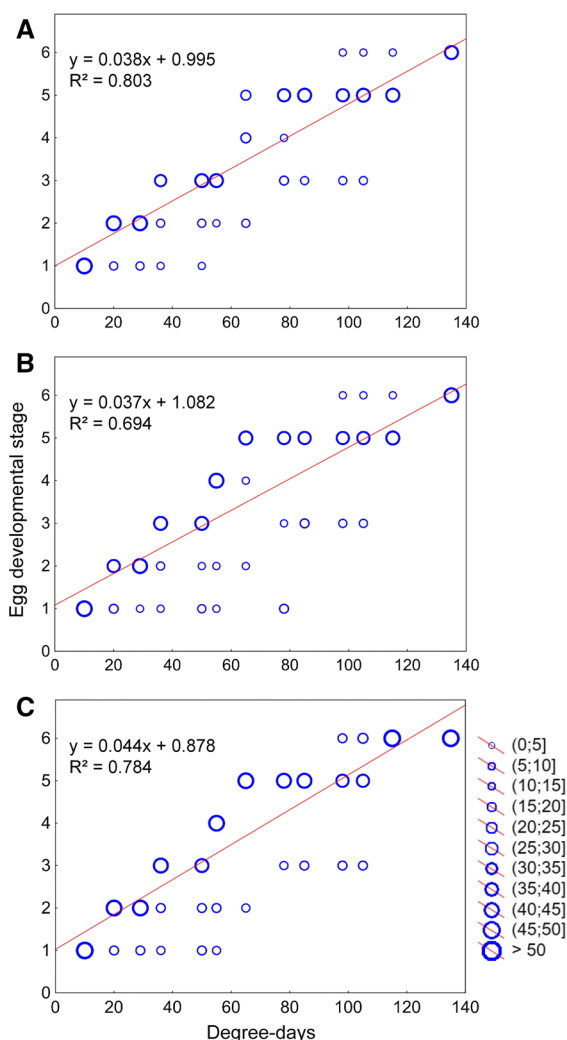
The effect of salinity on the rate of pike egg development (i.e., slopes of linear regression lines fitted to the egg development stage at 0 DD data) (Fig. 8) was statistically significant (ANCOVA,  $P < 0.001$ ) (Table 2). The differences in slopes reflect the faster egg development rate at 0 PSU than at 4 PSU.

Pronounced statistically significant differences among the three salinities were observed in successful hatches (89.7%—at 0 PSU; 19.7%—at 4 PSU; and 0.0%—at 7 PSU; Table 3) (Kruskal–Wallis,  $P < 0.05$ ). The differences among salinity (Kruskal–Wallis,  $P < 0.05$ ) and between 0 and 7 PSU (post hoc Dunn–Bonferroni test,  $P < 0.05$ ) were statistically significant. The differences between 0 and 4 PSU and between 4 and 7 PSU were not statistically significant (post hoc Dunn–Bonferroni test,  $P = 0.54$ ).

## Discussion

### Egg development: comparison to freshwater population

The results of the present study on pikes from a freshwater population showed that egg development in the saline waters of the coastal Baltic Sea (7 PSU) would be unsuccessful. Although the salinity of 4 PSU, which might occur in river mouths, provided some likelihood of pike egg survival, the number of hatched larvae was low (20%). The number of publications providing results on egg development at different salinities for strictly freshwater populations, not anadromous populations, is limited. Although a



**Fig. 6** Differences in the number of eggs at individual stages of development (scale from 1 to 6, according to Fig. 1) on given sampling days (DD) at salinities of 0 (A), 4 (B), and 7 (C) after fertilization in fresh water (Experiment I). The size of the circles corresponds to the number of eggs (only live eggs were analyzed). Linear regression functions were fitted to describe egg development rates at given salinities

slight increase in water salinities of 1–2 PSU might have positive effects on egg development and hatching success, further increases in salinity are disadvantageous for pike eggs (Bonisławska, 2014; Kuznetsov et al., 2016). In a synopsis on northern pike biological data, Raat (1988) reported that freshwater pikes are unable to produce offspring at salinities above 7 PSU. Bonisławska (2014) presented results from an experimental study on significantly increased freshwater

**Table 3** Hatching success of pike larvae after egg incubation in salinities of 0, 4, and 7 PSU (three replicates)

Experiment	Hatching success (mean $\pm$ SD, %)		
	Salinity 0 PSU	Salinity 4 PSU	Salinity 7 PSU
Exp. I— fertilization in fresh water	80.0 $\pm$ 2.0	83.3 $\pm$ 2.5	93.7 $\pm$ 2.5
Exp. II— fertilization in a given salinity	89.7 $\pm$ 3.1	19.7 $\pm$ 1.8	0 $\pm$ 0

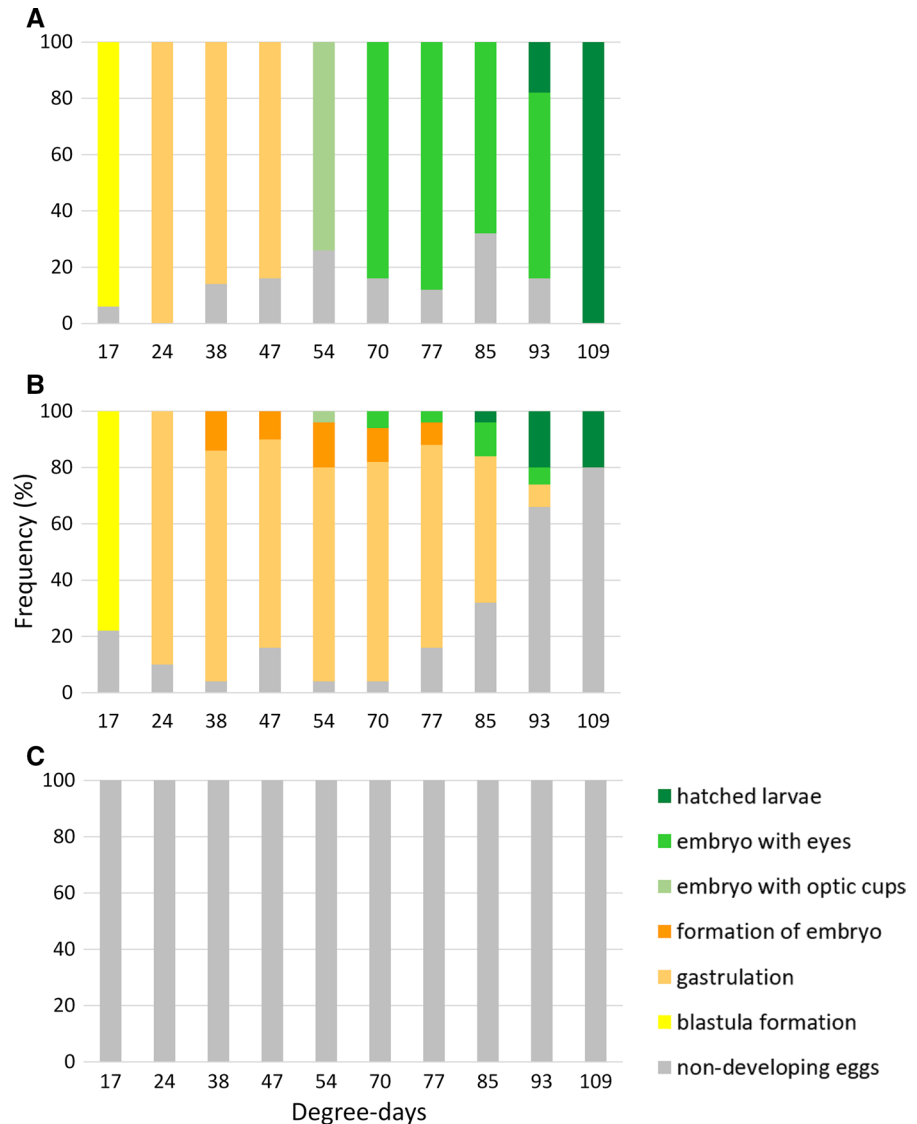
There were two variants of egg fertilization: in fresh water – eggs transferred after 1 h to experimental salinities (Experiment I), and directly in a given salinity (Experiment II)

pike embryo mortality and decreased hatching success at salinities higher than 2 PSU (hatching success at 3, 4, and 5 PSU was 29.2, 17.4, and 3.5%, respectively). At the same time, the number of deformed larvae at the hatching stage increased from 4.8% at 2 PSU to 24.6 at 3 PSU and 40% at 5 PSU. Thus, our results confirm previously reported information on significantly increased freshwater pike egg mortality starting from salinities of approximately 3 PSU and on the upper limit for their development at salinities of approximately 5 PSU.

#### Egg development: comparison to anadromous population

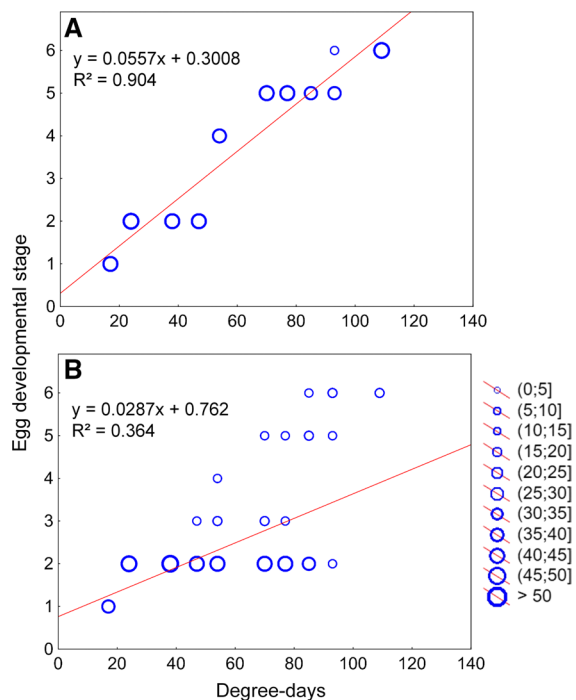
Our results can also be compared to other studies on the freshwater spawning of anadromous pike populations in the Baltic Sea. For pikes collected in freshwater streams and coastal waters of Kalmar Sound and Gotland Island, Westin & Limburg (2002) reported that a salinity of 6 PSU was the barrier for anadromous populations but that egg development was successful at 5 PSU. Although these authors did not provide detailed information on the proportion of hatching eggs, their results were generally consistent with ours. A significant difference in the number of developing eggs in the anadromous pike population from Kalmar Sound was noted between salinities of 0 and 3 PSU, while differences were higher between salinities of 0 and 5, 7, and 9 PSU (Sunde et al., 2018). Interestingly, an approximately 6% hatching success was observed

**Fig. 7** Comparison of pike egg development in Experiment II (fertilization at one of the three salinities analyzed) on given sampling days (DD) at salinities of 0 (A), 4 (B), and 7 (C). Fifty eggs were analyzed from each of 10 samplings



even at 7 and 9 PSU. As mentioned previously, our results showed no egg development at a salinity of 7 PSU. In comparison with the present study, the higher resistance of pike eggs to increased salinity that Sunde et al. (2018) reported could have resulted from different adaptations between anadromous and freshwater specimens. In particular, they reported that the subpopulation they studied was exposed in its natural spawning habitat to occasional brackish water inflows. Moreover, the subpopulation occasionally exposed to increased salinities exhibited a significantly greater tolerance to high salinity with hatching success at 7–9 PSU of approximately 35%

than did the subpopulation that spawned naturally in a stable, freshwater habitat. Sunde et al. (2018) indicated that some differences in egg development success in saline water could exist among anadromous populations in different areas of the Baltic Sea. Analogically, differences between anadromous and strictly freshwater populations, such as the one studied in this work, can also be expected. Vetemaa & Saat (1996) provided support for this theory in their study, which indicated that the eggs of ruffe, *Gymnocephalus cernuus* (Linnaeus, 1758), which is primarily a freshwater species, from a brackish population hatched at higher salinities (10 PSU)



**Fig. 8** Difference in the number of eggs at individual stages of development (scale from 1 to 6, according to Fig. 1) on given sampling days (DD) at salinities of 0 (**A**) and 4 (**B**) after fertilization in saline water (Experiment II). Circle size corresponds to the number of eggs (only live eggs were analyzed)

than did eggs from a freshwater population (below 8 PSU).

The mechanisms of the negative impact of salinity on embryonic development vary depending on fish species and salinity levels, and they can impact different periods of embryogenesis. For example, increased salinity can affect sperm mobility, eggshell permeability, or the development of the perivitelline space (Bonisławska, 2014). In the present experiment, normal egg development at 4 PSU was observed for up to 54 DD, after which most eggs ceased development at the gastrulation stage. Similar salinity results affecting egg development before the embryonic phase were presented by Jørgensen et al. (2010) for pike and by Vetemaa & Saat (1996) for ruffe. The most likely explanation in the case of pikes seems to be the potential effect of saline water on eggshell permeability resulting in disturbed ion transfer inside the eggs (Bonisławska, 2014).

### Egg development: comparison to resident brackish population

Although salinity above approximately 2–3 PSU significantly affects egg development and the hatching success of both anadromous and freshwater pike populations, this is not the case for resident brackish populations that successfully reproduce in waters with salinities above 6 PSU. Jørgensen et al. (2010) reported on a resident brackish pike population from Stege Nor (Denmark), and their eggs developed normally at salinities of 6 and 8.5 PSU, while at the lower salinities of 0 and 3, PSU development was abnormal and led to zero hatching success. Similar results for resident brackish populations were reported by Westin & Limburg (2002), who observed normal embryonic development at 6.6 and 6.9 PSU. Although the occurrence of resident pike populations has been described in numerous Baltic coastal areas, there is no sign of them in Polish coastal waters, including Puck Lagoon. However, historical information about the location of a pike protection area in Puck Lagoon (redrawn from historical map from year 1920) (see Fig. 1) suggests that a sea-spawning population might have inhabited this location in the past. Unfortunately, it is impossible to verify this theory.

### Egg development: inflow of saline waters into freshwater wetlands

In the present study, embryonic development was also observed when eggs were fertilized in fresh water and transferred to saltwater after 1 h. The eggs developed normally not only in a salinity of 0 but also in salinities of 4 and 7 PSU. In fact, hatching success was the highest at a salinity of 7 PSU and was higher at 4 PSU than at 0 PSU. Thus, it can be concluded that as soon as fertilization is successful and embryonic development is initiated, higher salinities are not only not harmful but might even have a positive effect on freshwater pike egg development and hatching success. This situation could occur under natural conditions, for example, when freshwater marshes (both natural and restored) are flooded during wind-driven backflows of brackish water into the mouths of rivers or streams. This conclusion is, of course, limited to geographical areas in which the salinity of coastal waters is not higher than 7 PSU. In the western Baltic, for example, on the Danish coast, inflows of highly

saline water of 10 PSU and greater are natural phenomena (Jørgensen et al., 2010). No other data on egg development in such an ecological scenario are available for comparison.

Using freshwater population in the current study referring to coastal waters

To some readers, it may be confusing that the current study of the environmental impact of Puck Lagoon (i.e., Baltic Sea coastal waters) on pike spawning was conducted using spawners from freshwater populations. The reason for this was the lack of any established anadromous populations in this geographical area. Natural reproduction has been limited since the 1970s because of restricted access to suitable freshwater spawning habitats. River regulation eliminated wetlands, and hydrological constructions such as pumping stations blocked access to shallow parts of two local rivers (see map in Fig. 1). Even if some individuals occasionally find their way to the main corridors of local rivers, these no longer provide appropriate habitats for pike spawning since they lack shallow, sheltered areas with slow currents and vegetation suitable for egg deposition. The magnitude of such natural spawning is extremely limited, which is evidenced by the pike catch data presented in this study. The existence of the Puck Lagoon pike population is possible mostly due to occasional stocking performed with fry obtained from freshwater populations. This is why the question of whether pike reproduction is successful in the saltwater environment of Puck Lagoon must be answered with experiments on eggs obtained from freshwater spawners.

#### Conclusion and suggested management strategies

The extremely low pike population level in Puck Lagoon raises questions about which management strategies should be undertaken. As the results of our study revealed, the ability of the local population, which is more freshwater in nature than anadromous, to spawn in the saline waters of the bay is negligible. At the same time, natural pike spawning in fresh water is currently almost impossible because of the lack of suitable spawning grounds. Stocking might be a way to improve the situation, but only in the short-term (see Fig. 2) unless a long-term stocking program is developed. However, a long-term stocking program

conducted in Danish waters was unsuccessful, probably because of the low survival of the freshwater fry that were released (Larsen et al., 2005; Jacobsen et al., 2008) into waters that had a much higher salinity (approximately 8–10 PSU) than that in Puck Lagoon (approximately 6–7 PSU). Thus, presumably a long-term stocking program in Puck Lagoon could be successful. However, with the Danish experience in mind, acclimatizing fry to saline waters during rearing before their release as stocking material must also be considered.

The most promising solution, however, seems to be restoring spawning grounds by introducing so-called pike factories, as has been done in Sweden and Denmark (Nilsson et al., 2014; Engstedt et al., 2017). Engstedt et al. (2017) provided an extended review of pike spawning habitat restoration. In general, pike factories are a more natural way of restoring spawning grounds compared to the popular North American rearing marshes into which adult pikes are stocked to spawn (Fago, 1977). The present study revealed that the survival of eggs was good in saline waters if they were fertilized and embryonic development began in fresh water; thus, introducing pike factories could be a successful solution. The results of the current study indicate that it would be possible even to locate pike factories in areas directly adjacent to lagoons and bays despite the risk of flooding with saline waters. In fact, an increased salinity of up to 7 PSU just one hour after fertilization was beneficial for hatching success.

One more management possibility would be to introduce a resident brackish pike population by releasing this type of fry stocking material into Puck Lagoon and other similar habitats in Baltic Sea coastal areas. There are indications that such a population inhabited Puck Lagoon in the past. This strategy of introducing a resident brackish pike population to restore pikes to Baltic Sea habitats has already been suggested by Lappalainen et al. (2008) as a parallel solution to the creation of pike factories.

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**Data availability** The datasets analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors have no potential conflicts of interest to declare.

**Ethical approval** No animal ethics committee approval and no other specific permissions were required to carry out this study.

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## OŚWIADCZENIE

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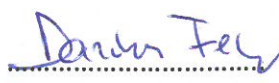
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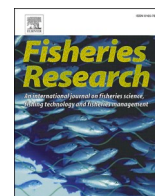


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# Effects of temperature on somatic growth, otolith growth, and uncoupling in the otolith to fish size relationship of larval northern pike, *Esox lucius* L

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## ABSTRACT

Verifying the link between somatic and otolith growth is crucial for a number of analyses that provide data on the ecology of early life stages of fish. In the current study, sagittal and lapillar otoliths were extracted from larval northern pike ( $n = 720$ ) that were reared from eggs in RAS systems at three temperatures (10 °C, 15 °C, and 22 °C) for 28 days. The growth rate (SL at age) of larval northern pike was significantly dependent on rearing temperature with the highest rate observed at 22 °C (0.83 mm·d<sup>-1</sup>), a lower rate at 15 °C (0.48 mm·d<sup>-1</sup>), and the lowest at 10 °C (0.25 mm·d<sup>-1</sup>). The maximum value of growth observed for an individual larvae at a certain age was 1.28 mm·d<sup>-1</sup>. The higher somatic growth rate at higher temperatures was followed by faster lapillus and sagitta linear growth rates. Positive relationships between otolith growth and somatic growth were noted not only among temperatures but also within given temperatures. The sizes of lapilli and sagittae were strongly correlated with fish size, but both somatic growth and temperature had a statistically significant effect on those relationships. Magnitude of this phenomenon was however fish size dependent. We concluded that larval northern pike otoliths (both sagittae and lapilli) are a reliable source of information on somatic growth, and they can be used for increment trajectory width analysis and marginal otolith increment width analysis. However, if growth back-calculation is to be employed, it is recommended to consider the somatic growth and temperature effect on the fish size-otolith size relationship.

## 1. Introduction

Northern pike (*Esox lucius*) is a species of significant importance to both commercial and recreational fisheries that inhabits a wide range of environments where, during its early life stages, it encounters temperatures from 6 to above 20 °C (Craig, 2008). However, temperatures above 22 °C (the maximum value used in the present study) are very occasional. Unfortunately, this species is close to extirpation in many geographical areas including freshwater and coastal marine systems, for example the Baltic Sea (Skov and Nilsson, 2018). Although over-exploitation can explain this to some extent, the disappearance of suitable spawning grounds and low recruitment seem to be the main reasons for the decline of population size of this species (Nilsson et al., 2014; Larsson et al., 2015; Skov and Nilsson, 2018; Fey et al., 2019). Other factors related to human activities in coastal areas, such as wind farms and underwater cables, did not affect the growth or survival of the early life stages of northern pike (Fey et al., 2019) despite its demersal eggs and larvae attached to the substrate during the yolk-sac stage (Horbowa and Fey, 2013). The more endangered the northern pike

populations are, the more urgent it is to better understand ecological relations during the early life period. This is especially true since the recruitment success and states of given fish populations are frequently determined during the larval and juvenile life stages (Houde, 1987) when a number of factors are responsible for mortality in given populations (Anderson, 1988).

The main source of information on the early life history of fish are otoliths – calcified structures used by fish for balance, hearing, and orientation (for reviews on otolith formation and daily increment deposition see Morales-Nin, 1992, 2000) – that can provide data on, *inter alia*, age, growth, condition, hatching dates, geographical area of origin, and mortality at individual and population levels in larvae (Campana and Nielson, 1985; Francis, 1990; Secor and Dean, 1992; Campana and Jones, 1992; Campana, 2005; Berg et al., 2017), specimens that are several months old (Fey and Linkowski, 2006), and even adults (Hussy et al., 2010). Bearing in mind all the advantages of otolith methods, one should also consider the complexity of processes affecting short- and long-term otolith growth and otolith microstructures (e.g., response time, differences in how body size changes are reflected in length and

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mass). This is especially well visualized in studies that assess the somatic growth of larval fish using several methods simultaneously such as RNA/DNA, condition factor K, and otolith increment widths (Peck et al., 2015), or, for example, RNA/DNA and marginal otolith increment widths (Do Souto, 2019a).

Before otolith methods can be applied to obtain biological data for ecological studies, it is important to evaluate several assumptions. First, when the first increment is formed and whether increments are deposited daily must be verified (Geffen, 1992; Campana, 2001). Some factors such as temperature and somatic growth should be considered as they affect increment formation and visibility under the microscope. Otolith increments can be too narrow to be distinguished under a light microscope if somatic growth is too slow, for example as a result of poor feeding conditions (Geffen, 1982; Folkvord et al., 2000; Fox et al., 2003) or low temperatures (Radtke and Fey, 1996; Fukuda et al., 2009). On the other hand, even if increments are formed daily and are above the resolution of light microscope, significant differences in their enumeration may occur among readers with different experience, resulting in substantial error in growth rate and hatch-dates of larval fish estimates (Spich and Fey, 2020). Moreover, it should be confirmed if the otolith size-fish size relationship is independent of somatic growth and temperature and if otolith growth follows somatic growth (Geffen, 1992) so that increment widths can be used as proxies for fish growth on given days. Results obtained for species other than northern pike indicate that slower growing specimens might have relatively (i.e., at the same fish size) larger otoliths (Mosegaard et al., 1988; Secor and Dean, 1989; Fey, 2001; 2006), and otolith growth can also be affected not only by, as expected, somatic growth but also independently by temperature itself (Mosegaard et al., 1988; Barber and Jennings, 2001; Fey, 2001, 2005, 2006; Fey and Hare, 2012). In such cases, when other factors control these two relationships, it can be concluded that uncoupling in otolith size-fish size relationship and/or fish growth-otolith growth occurred (Mosegaard et al., 1988).

The uncoupling in somatic growth-otolith growth and otolith size-fish size relationships can have particularly serious consequences for the accuracy of results obtained with methods based on increment width measurements, like growth back-calculation (Campana, 1990; Francis, 1990; Vigliola and Meekan, 2009; Ashworth et al., 2017; Morrison et al., 2019), marginal otolith increment width analysis (e.g., Sepúlveda, 1994; Fey, 2001, 2005; Do Souto et al., 2019a) or increment width trajectory analysis (e.g., Gultiérrez and Morales-Nin, 1986; Paperno et al., 1997; Do Souto et al., 2019b).

The assumption of the daily periodicity of increment formation in northern pike otoliths and the timing of the first increment formation were confirmed previously for the sagitta (Fey et al., 2018) and lapillus (Wang and Eckmann, 1992; Fey et al., 2018). However, there is still no data to support the assumption that otolith growth depends on somatic growth or that the fish size-otolith size relationship is independent of somatic growth. Confirmation of these assumptions is especially important for early life stages of species like northern pike that are characterized by fast growth and that possibly experience wide ranges of temperature (Skov and Nilsson, 2018).

The aim of the present study was to determine for larval and early juvenile northern pike (9–27 mm SL) the effect of somatic growth and temperature (10, 15, 22 °C) on the otolith growth (sagittae and lapilli). It was also verified whether the otolith size to fish size relationship was independent from the effects of somatic growth.

## 2. Materials and methods

### 2.1. Experimental conditions

The experiments were performed in nine tanks with volumes of 55 L each that were divided into three freshwater recirculating aquaculture systems (RAS) at three temperatures (mean ± SD) of 10 ± 0.6 °C, 15 ± 0.4 °C, and 22 ± 0.5 °C. There were three replicates for each

temperature (3 temperatures x 3 replicates). Each of the systems was equipped with UV sterilization and a filtering-deposit container with a volume of 70 L providing both mechanical and biological filtration. The volume of the biological filtration in each of the recirculating systems (3 tanks each) was 30 L, and the water flow for each of them was set to ca. 150 L/h. A roll of green plastic net (mesh size 0.5 cm, 20 cm in length x 8 cm in diameter) was placed in each of the tanks as substitute bottom substrate. The color of the tank bottoms was grey, and the tank walls were made of glass and were not covered. The tanks were located in a completely dark room in which light conditions (12 h light/12 h dark, with light on from 08:00 to 20:00) were regulated automatically. Temperature was measured three times daily with an electronic temperature reader. The oxygen level during the experiment was measured daily and was 7.8 ± 0.9 mg·L<sup>-1</sup> (mean ± SD) for all the tanks throughout the experimental period. The total time of the experiment was 28 days.

### 2.2. Fish larvae rearing

Eyed-egg northern pike embryos were transported to the laboratory from a commercial hatchery. The offspring of several males and females was randomized with respect to parentage. Three thousand eggs were placed in each of nine bags used for transportation. Mass hatching occurred during transport, and the hatched larvae were placed in tanks (the contents of one bag in one tank). Hatching success ranged from 92 to 95 % in all of the bags transported. The temperature was 10 °C in each tank at the beginning of the experiment. The temperature was then increased to 15 °C within 24 h in one of the systems, and to 22 °C within 48 h in another. These temperatures were maintained constant thereafter. Following yolk-sac absorption, feeding was conducted *ad libitum* every 1.5 h during periods with light with specialist fodder, as follows: ArtEX 2 (0.15 mm) (Aller Aqua) for the first 4 days after yolk-sac absorption; Perla Larva Proactive 4.0 (0.3–0.5 mm) (Skretting AS) from day 3 after yolk-sac absorption periods. Dead fish, unconsumed fodder, and excrement were cleaned from the tank bottoms every morning by siphoning.

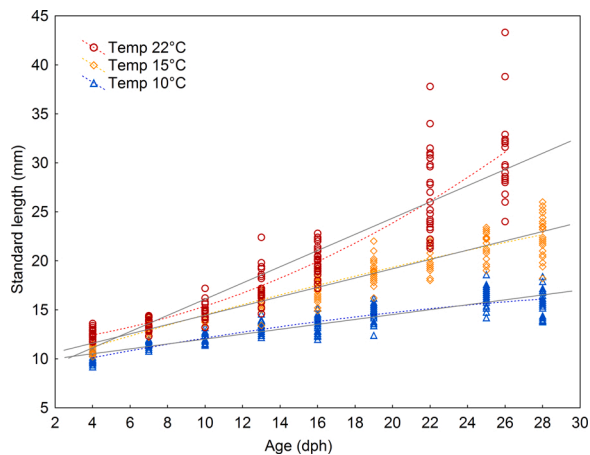
### 2.3. Otolith analysis

A sample of 30 specimens (10 specimens from each of the three replicates) was collected from each temperature 4, 7, 10, 13, 16, 19, 22, 25–26, and 28 days after hatching and placed in vials filled with 96 % alcohol. The alcohol was changed one week later since Fey (2018) showed that even alcohol cannot prevent otoliths from becoming damaged or errors occurring during otolith size measurements and subsequent fish growth rate calculations. Three samples (two from 22 °C and one from 10 °C), 30 specimens each, were lost because of the alcohol evaporating from the vials. Otoliths (left and right sagittae and lapilli) were extracted from each larva a few weeks later and placed on microscope slides, distal surface down (sulcus up) and covered with DEPEX mounting medium (Electron Microscopy Sciences, Hatfield, PA). The standard length (SL) of each larva was measured to the nearest 0.1 mm when the otoliths were extracted. All the SL measurements were corrected for shrinkage before further analysis by adding 0.7 mm (Greszkiewicz and Fey, 2018). The sizes (i.e., maximum diameters) of the left and right sagittae and lapilli were measured using an image analysis system (Image-Pro Premier, Media Cybernetics, Inc., Rockville, MD) under an Eclipse 80i transmitted light microscope (Nikon Corp., Tokyo, Japan). In total, 720 sagittae and lapilli were measured. The mean values of maximum diameter measurements from the left and right otoliths were used for further analysis.

### 2.4. Data analysis

The differences in SL among three tanks within each age group (i.e., day of experiment) were estimated for each temperature separately with the ANOVA mixed effects model, where age was the constant and tank





**Fig. 1.** Standard length (SL) at age of larval northern pike, *Esox lucius*, during the first 28 days of life under controlled conditions at three temperatures of 10, 15, and 22 °C. The slope of the fitted linear regressions indicated mean growth during the analysed period. Dotted lines indicate best-fit nonlinear regressions used for residual analysis for individual larvae to describe them as growing above or below the mean for a given temperature group: 10 °C,  $y = -0.0048 \cdot x^2 + 0.4018 \cdot x + 8.5821$ ,  $R^2 = 0.865$ ; 15 °C,  $y = 0.4782 \cdot x + 9.6008$ ,  $R^2 = 0.913$ ; 22 °C,  $y = 0.0225 \cdot x^2 + 0.1751 \cdot x + 11.381$ ,  $R^2 = 0.871$ .

the random factor. Since for all age groups the effect of tank was insignificant ( $P > 0.05$ ), the data were merged among tanks.

Linear regression functions were fitted to SL at age data separately for 10, 15, and 22 °C to assess mean larva growth at these temperatures. Correspondingly, otolith size at age data were described with linear functions to estimate otolith growth. The slopes describing mean larval growth rates at the three temperatures analysed were then plotted against the slopes describing their otolith growth rates in order to evaluate the effect of temperature on the otolith growth increase in proportion to somatic growth increase.

Additionally, the mean somatic growth rate (GR) was calculated for each larva with the equation  $GR = (SL_c - SL_h) (\text{age in days})^{-1}$ ; where:  $SL_c$  = standard length at catch (mm);  $SL_h$  = standard length at hatch (mm). The size of the larvae at hatching was assumed to be 9 mm SL (Horbowa and Fey, 2013). Although using the mean value of size at hatch introduced some inaccuracy in the estimates of the GR of individuals, the overall result for all larvae together reflected the reality of the GR range of the different temperatures. Because using the same mean size at hatch for all individuals introduced the highest inaccuracy for the smallest larvae, these results are presented with the exclusion of 4-day-old larvae, i.e., starting from 7-day-old larvae.

The statistical significance of the effects of temperature (grouping

factor) and age (covariate) on SL, otolith size, and GR of individual larvae (dependent variables) were evaluated with General Linear Model (GLM). If the GLM equal slopes model indicated the significance of interaction between grouping factor and covariate (i.e., significant differences in slopes of the regression lines describing the analysed relationship), the test was repeated with GLM unequal slopes model to obtain final statistics for the grouping factor overall effect. If the interaction term was insignificant (i.e., slopes were not different), Analysis of Covariance (ANCOVA) was conducted to indicate the significance of grouping variable effect (i.e., differences between intercepts).

Furthermore, the relationship between larvae and otolith growth rates was evaluated at each of the temperatures for both the sagittae and lapilli with residual analysis of SL at age and otolith size at age relationships. These residuals (converted from mm and  $\mu\text{m}$  to % of the corresponding means) were obtained after the data were described with nonlinear best-fit functions (GLM, see Figs. 1 and 3, for the formulas). Although the earlier fitted linear regression lines were accurate enough to describe the mean growth during first month of life, for both SL and otolith size, the residual analysis required high precision for individuals so as to describe both larvae and otoliths as fast growing (above mean, positive residuals) or slow growing (below mean, negative residuals). The residuals data were described with linear regressions to indicate the significance and strength of those relationships.

The otolith size-fish size relationship was described for all larvae pooled together, but the data were divided into: 1) two groups in respect to GR of the individual larvae – fast growing (above mean, positive residuals of the SL at age function) and slow growing (below mean, negative residuals of the SL at age function). The SL at age function used for residuals calculation was fitted to all temperature data pooled ( $SL = 9.4843 + 0.4622 \cdot x$ ;  $R^2 = 0.506$ ,  $P < 0.001$ ); and 2) three groups in respect to temperature (10, 15, and 22 °C). The effect of GR and temperature on the relationship between SL and otolith size was evaluated with GLM equal slopes model (comparison of slopes). The GLM unequal slopes model (final statistics for grouping factor effect) was then used if the slopes were statistically different, or the ANCOVA (comparison of intercepts) was used if the slopes were not significantly different. The statistical comparison of GR and temperature effect on otolith size-fish size relationship was conducted only for larvae representing the same SL range between GR groups and among temperature groups.

The data were analysed with Statistica, vers. 12.0 (TIBCO Software, Inc., Palo Alto, CA). Differences were considered statistically significant at  $P < 0.05$  ( $\alpha = 0.05$ ).

### 3. Results

#### 3.1. Temperature effect on somatic growth

The mean growth rate (slopes of the regression lines fitted to SL at

**Table 1**

Results of linear regression analysis of standard length (SL) at age, and otolith size (sagittae maximum diameter, SMD; lapilli maximum diameter, LMD) at age of larval pike during the first 28 days of life at three temperatures.

Group	Linear regression	n	R <sup>2</sup>	P	Growth Rate
<i>Somatic growth</i>					
10 °C	SL = 9.5191 + 0.2486 Age	240	0.853	$P < 0.001$	0.25 mm·d <sup>-1</sup>
15 °C	SL = 9.6008 + 0.4782 Age	270	0.916	$P < 0.001$	0.48 mm·d <sup>-1</sup>
22 °C	SL = 7.7078 + 0.8313 Age	210	0.852	$P < 0.001$	0.83 mm·d <sup>-1</sup>
<i>Sagittae growth</i>					
10 °C	SMD = 52.0581 + 5.5189 Age	240	0.901	$P < 0.001$	5.5 $\mu\text{m} \cdot \text{d}^{-1}$
15 °C	SMD = 54.2136 + 10.9575 Age	270	0.948	$P < 0.001$	10.9 $\mu\text{m} \cdot \text{d}^{-1}$
22 °C	SMD = 12.0831 + 20.6308 Age	210	0.916	$P < 0.001$	20.6 $\mu\text{m} \cdot \text{d}^{-1}$
<i>Lapilli growth</i>					
10 °C	LMD = 65.1512 + 3.0096 Age	240	0.853	$P < 0.001$	3.0 $\mu\text{m} \cdot \text{d}^{-1}$
15 °C	LMD = 75.6637 + 4.979 Age	270	0.942	$P < 0.001$	4.9 $\mu\text{m} \cdot \text{d}^{-1}$
22 °C	LMD = 70.9934 + 7.0395 Age	210	0.917	$P < 0.001$	7.0 $\mu\text{m} \cdot \text{d}^{-1}$

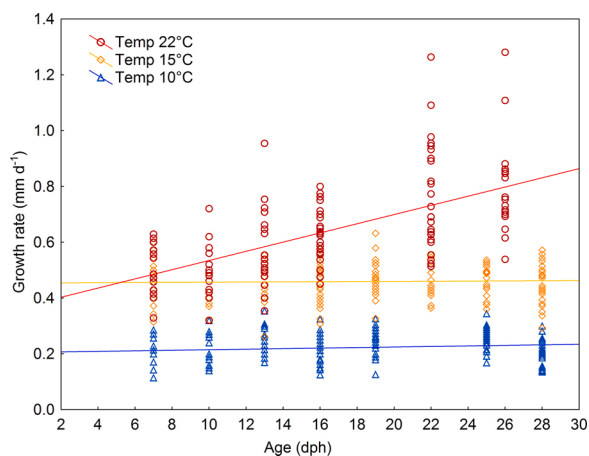
**Table 2**

Results of GLM (unequal slopes model) analysis for the effect of temperature (grouping variable) and age (covariate) on standard length, otolith size, and growth rates (dependent variables) of individual larvae of pike during the first 28 days of life. Three temperatures were used: 10, 15, and 22 °C.

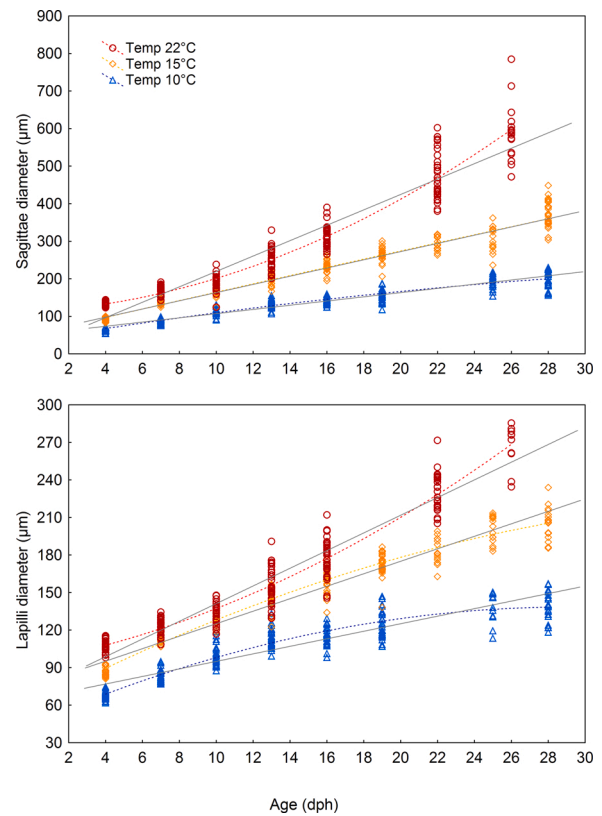
	SS	df	MS	F	P
<b>Standard length</b>					
Intercept	10.993	1	10993	4104	$P < 0.001$
Temp* Age	10826	3	3608	1347	$P < 0.001$
Temp	101	2	50	18	$P < 0.001$
Error	1743	714	2		
<b>Growth rates of individual larvae</b>					
Intercept	13.94	1	13.59	1197	$P < 0.001$
Temp* Age	1.74	3	0.58	49	$P < 0.001$
Temp	3.62	2	1.81	155	$P < 0.001$
Error	7.57	714	0.01		
<b>Sagittae maximum diameter</b>					
Intercept	212691	1	212691	234	$P < 0.001$
Temp* Age	6191441	3	2063814	2275	$P < 0.001$
Temp	49529	2	24764	27	$P < 0.001$
Error	582152	624	907		
<b>Lapilli maximum diameter</b>					
Intercept	622815	1	622815	3837	$P < 0.001$
Temp* Age	1069470	3	356490	2196	$P < 0.001$
Temp	2841	2	1420	8	$P < 0.001$
Error	103708	714	162		

age data) of larval northern pike depended significantly on rearing temperature (Fig. 1, Table 1), with the highest rate observed at 22 °C (0.83 mm·d<sup>-1</sup>), a lower rate at 15 °C (0.48 mm·d<sup>-1</sup>), and the lowest at 10 °C (0.25 mm·d<sup>-1</sup>). The described above among temperatures differences in slopes of the SL at age regressions (i.e., somatic growth) were statistically significant (GLM unequal slopes model, all  $P < 0.05$ , Table 2). The growth increase with temperature (slope of a linear function fitted to the mean growth rates at the temperatures presented above) corresponded to 0.05 mm·d<sup>-1</sup> per 1 °C.

The GR of individual larvae (Fig. 2), however, reached much higher values, up to 1.28 mm·d<sup>-1</sup>, than the means described above. The effect of both age and temperature on individual larval GR were statistically significant, but the interaction was significant as well (GLM unequal slopes model, Table 2). The GRs were independent of age in 10 °C ( $y = 0.205 + 0.001 \cdot x$ ;  $n = 210$ ;  $R^2 = 0.019$ ;  $P = 0.054$ ) and 15 °C ( $y = 0.454 + 0.0003 \cdot x$ ;  $n = 240$ ;  $R^2 = 0.001$ ;  $P = 0.687$ ) but were higher for relatively



**Fig. 2.** Mean growth rates of individual larvae of northern pike, *Esox lucius*, at three temperatures of 10 °C,  $n = 230$ , 15 °C,  $n = 260$ , and 22 °C,  $n = 200$ . Linear regressions were fitted to visualize the GR dependence on age. Regressions for 10 and 15 °C were not statistically significant ( $P > 0.05$ ), regression for 22 °C was statistically significant ( $P < 0.05$ ).



**Fig. 3.** Sagittae and lapilli maximum diameter at age of larval northern pike, *Esox lucius*, during the first 28 days of life under controlled conditions at three temperatures of 10, 15, and 22 °C. The slope of the fitted linear regressions indicate mean otolith growth during the period analysed. Dotted lines indicate best-fit nonlinear regressions used for residual analysis for individual larvae to describe their otoliths as growing at a rate above or below the mean for a given temperature group: sagittae 10 °C,  $y = -0.0806 \cdot x^2 + 8.1113 \cdot x + 36.25$ ; sagittae 15 °C,  $y = -0.0141 \cdot x^2 + 11.4031 \cdot x + 51.5585$ ; sagittae 22 °C,  $y = 0.6103 \cdot x^2 + 2.7995 \cdot x + 111.89$ ,  $R^2 = 0.936$ ; lapilli 10 °C  $y = -0.082 \cdot x^2 + 5.7983 \cdot x + 47.594$ ; lapilli 15 °C,  $y = -0.0531 \cdot x^2 + 6.8214 \cdot x + 64.0037$ ,  $R^2 = 0.947$ ; lapilli 22 °C  $y = 0.195 \cdot x^2 + 1.9743 \cdot x + 97.339$ .

older specimens in 22 °C ( $y = 0.3695 + 0.0165 \cdot x$ ;  $n = 180$ ;  $R^2 = 0.385$ ;  $P < 0.001$ ). The 22 °C group was moreover characterized by the highest GR variation in comparison to 10 and 15 °C.

### 3.2. Temperature and somatic growth effect on otolith growth

A positive temperature effect was observed in otolith growth (slopes of the fitted linear regression functions to otolith size at age data, Table 1) for both sagittae (22 °C: 20.6 µm d<sup>-1</sup>; 15 °C: 10.9 µm d<sup>-1</sup>; 10 °C: 5.5 µm d<sup>-1</sup>) (Fig. 3a) and lapilli (22 °C: 7.0 µm d<sup>-1</sup>; 15 °C: 4.9 µm d<sup>-1</sup>; 10 °C: 3.0 µm d<sup>-1</sup>) (Fig. 3b). The statistical significance of the differences in described above slopes (i.e., otolith growth) was confirmed with GLM unequal slopes model for both sagittae and lapilli (all  $P < 0.05$ , Table 2).

Sagittae and lapilli growth (slopes of regressions fitted to otolith size at age data) increased linearly with the somatic growth rate (slopes of regressions fitted to the SL at age data) within the full temperature range from 10 to 22 °C (Fig. 4).

The differences in otolith size and the microincrement pattern of sagittae and lapilli among pike larvae growing at temperatures of 10, 15, and 22 °C for 25-day-old individuals are presented in Fig. 5.

### 3.3. Somatic growth effect on otolith growth at given temperatures

The relationship between otolith growth and somatic growth was present not only among temperatures but also within a given

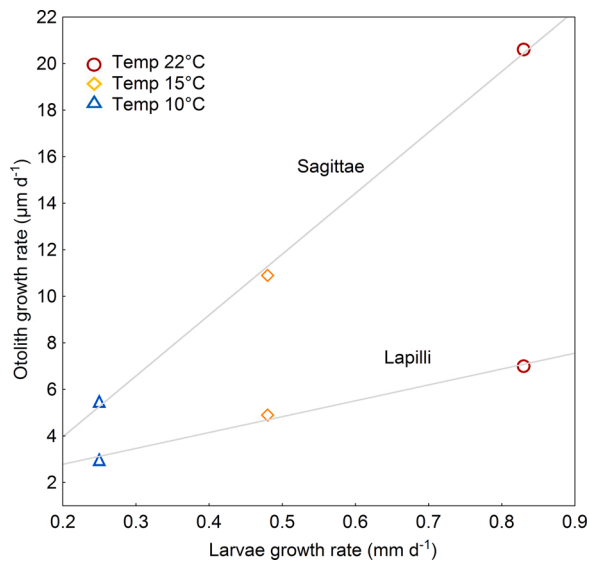


Fig. 4. The relationship between mean otolith growth rate and mean larval growth rate, for sagittae and lapilli of northern pike, *Esox lucius*, under controlled conditions at temperatures of 10, 15, and 22 °C (sagittae:  $y = -1.2763 + 26.1723 \cdot x$ ,  $R^2 = 0.998$ ; lapilli:  $y = 1.4187 + 6.823 \cdot x$ ,  $R^2 = 0.991$ ). The mean growth rates were estimated from the slopes of linear regression lines fitted to SL at age and otolith size at age data.

temperature, as was shown by the analysis of residuals of SL at age and otolith size at age relationships (Fig. 6). The linear regressions fitted to these data were statistically significant ( $P < 0.001$ ) for all three temperatures and two otolith types. The best fit was observed at the highest

temperature of 22 °C for both sagittae ( $10\text{ °C}$ ,  $y = 0.0237 + 0.9811 \cdot x$ ,  $n = 240$ ,  $R^2 = 0.357$ ;  $15\text{ °C}$ ,  $y = 0.4114 + 0.8911 \cdot x$ ;  $n = 270$ ;  $R^2 = 0.503$ ;  $22\text{ °C}$ ,  $y = 0.6259 + 0.9068 \cdot x$ ,  $n = 210$ ,  $R^2 = 0.728$ ) and lapilli ( $10\text{ °C}$ ,  $y = 0.0214 + 0.893 \cdot x$ ,  $n = 240$ ,  $R^2 = 0.406$ ;  $15\text{ °C}$ ,  $y = 0.2087 + 0.5781 \cdot x$ ;  $n = 270$ ,  $R^2 = 0.398$ ;  $22\text{ °C}$ ,  $y = 0.349 + 0.6401 \cdot x$ ,  $n = 210$ ,  $R^2 = 0.648$ ).

### 3.4. Otolith size-fish size relationship

The sizes of the sagittae and lapilli were strongly correlated with SL (Fig. 7). However, when the data were split into two groups, GR above mean (positive residuals of SL at age regression) and GR below mean (negative residuals of SL at age regression) (Table 3, Fig. 7a and b), there was statistically significant difference in slopes between the GR groups for both sagittae and lapilli (GLM,  $P < 0.05$ ; Table 4). This GR effect was fish size dependent (significant interaction term,  $P < 0.05$ ) and therefore it was not possible to describe this phenomenon in an unambiguous way. The GR effect was evaluated for larvae representing the same SL range in this comparison.

The data were also divided into three groups according to experienced temperatures: 10, 15, and 22 °C (Table 3, Fig. 7c and d). For sagittae, there was no statistically significant difference in slopes (GLM,  $P = 0.273$ ), but there was a difference in intercepts (ANCOVA,  $P < 0.05$ ; Table 4). The larvae from higher temperatures had relatively larger otoliths. For lapilli, the difference in slopes among the three temperature groups was statistically significant (GLM,  $P < 0.05$ ; Table 4), but there was no clear pattern of the temperature effect on the otolith size-fish size relationship for this otolith type (significant interaction term,  $P < 0.05$ ). The temperature effect was evaluated for larvae representing the same SL range in this comparison.

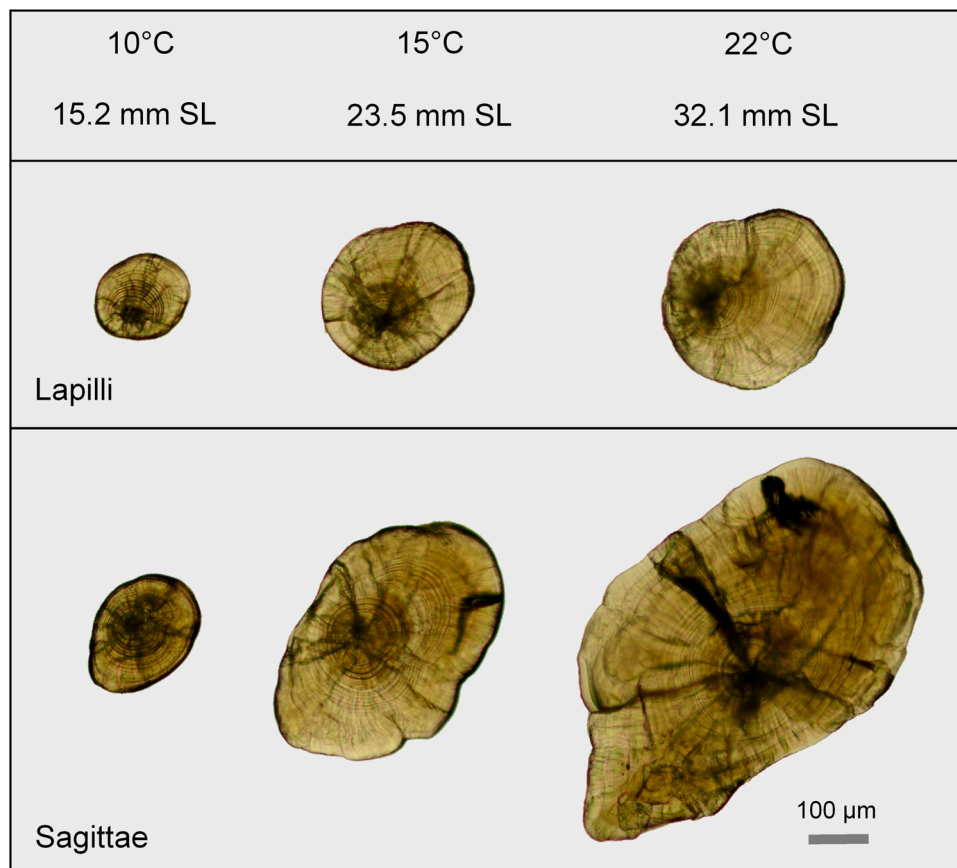
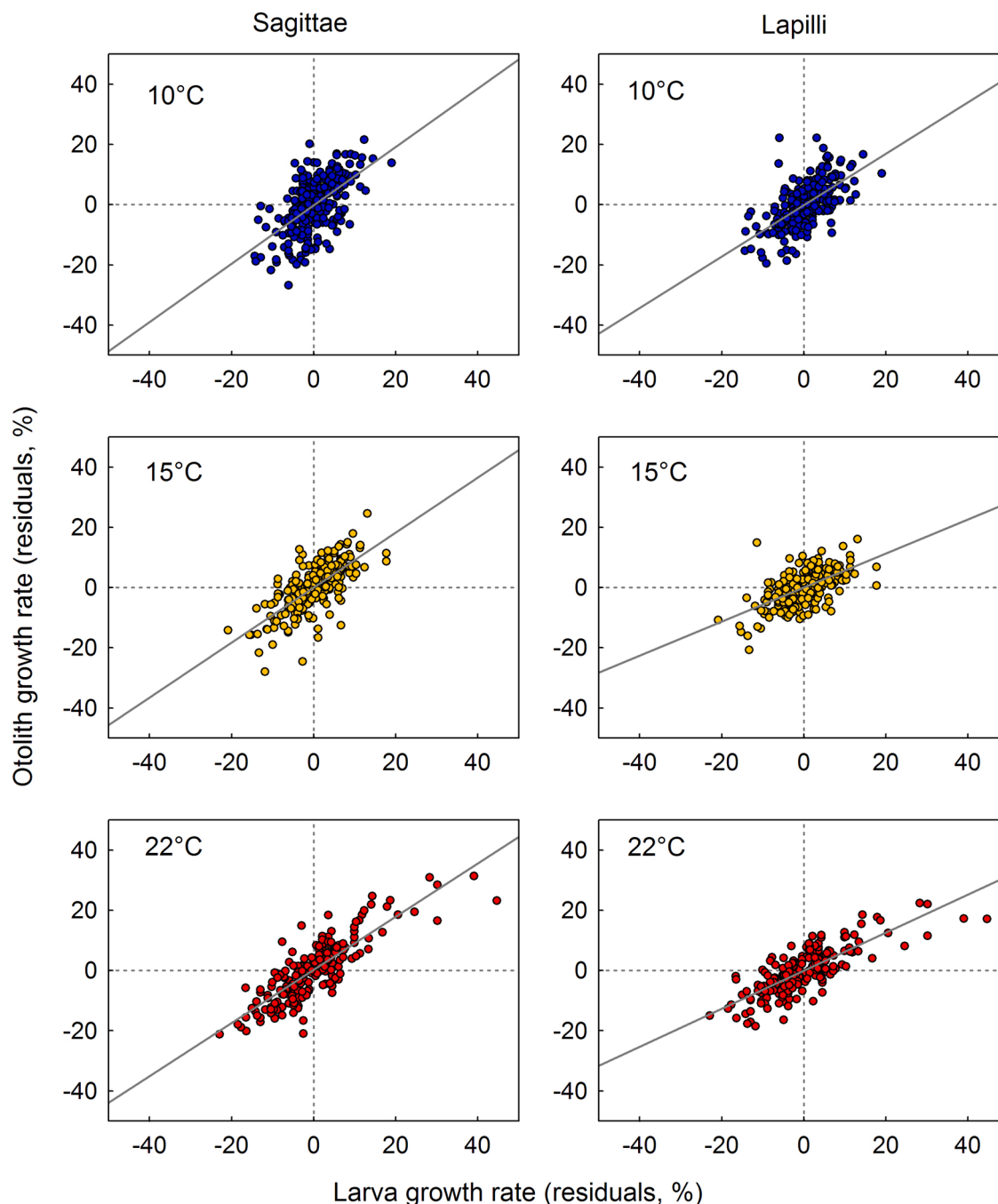


Fig. 5. Sagittae and lapilli of larval northern pike, *Esox lucius*, of the same age (25d) but growing at different temperatures of 10, 15, and 22 °C.





**Fig. 6.** The relationship between otolith growth rate and larval growth rate in sagittae and lapilli of northern pike, *Esox lucius*, under controlled conditions at temperatures of 10, 15, and 22 °C. Each point represents individual growth rate values estimated as residuals (%) from linear regression lines describing the SL at age and otolith size at age relationships within a given temperature.

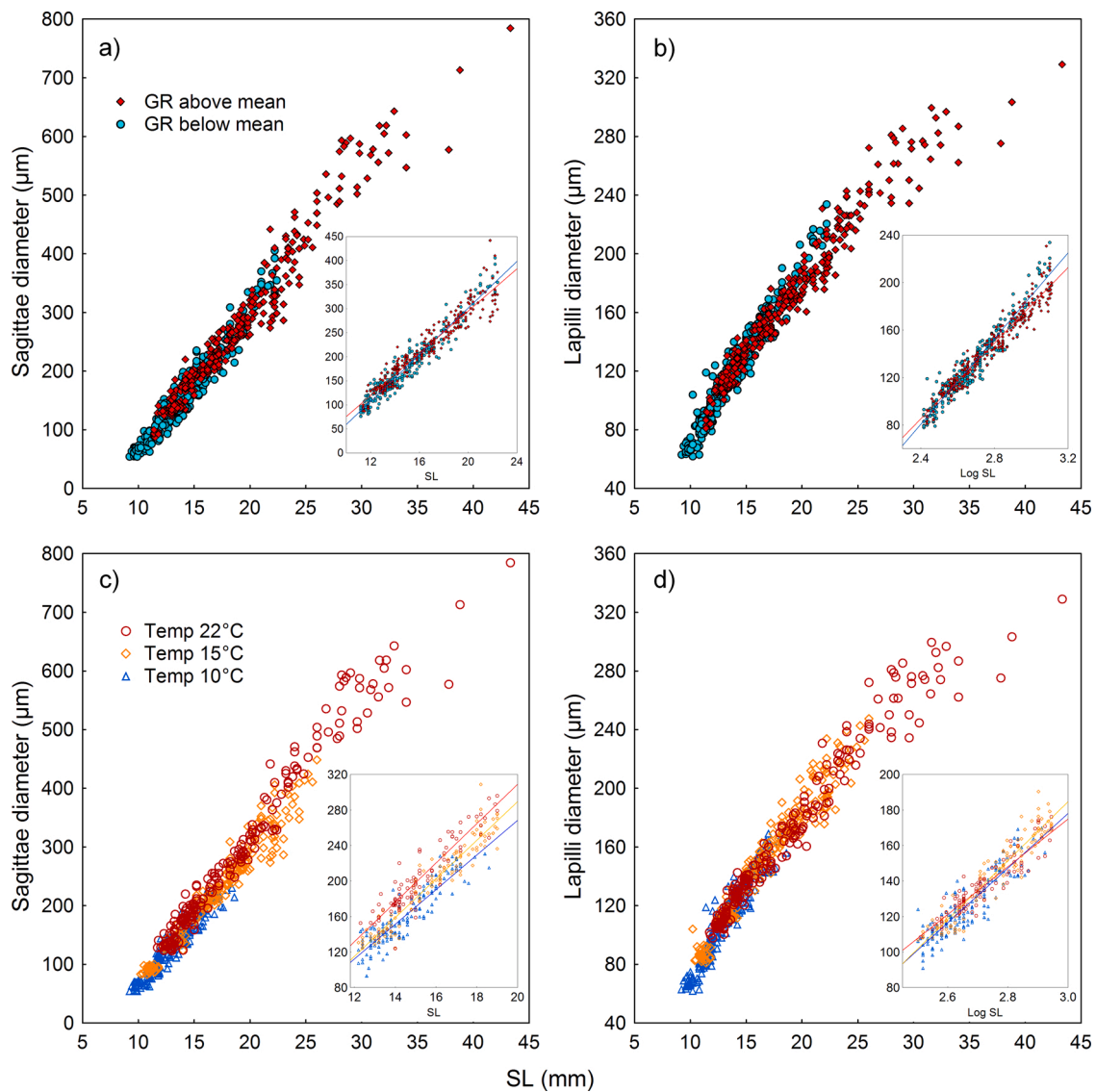
## 4. Discussion

### 4.1. Temperature effect on somatic growth

The growth rate of larval northern pike in the present study depended significantly on rearing temperature with the highest mean rate observed at 22 °C ( $0.83 \text{ mm}\cdot\text{d}^{-1}$ ), a lower rate at 15 °C ( $0.48 \text{ mm}\cdot\text{d}^{-1}$ ), and the lowest at 10 °C ( $0.25 \text{ mm}\cdot\text{d}^{-1}$ ). The mean life growth rates of individuals were even higher at up to  $1.28 \text{ mm}\cdot\text{d}^{-1}$ . These results were not surprising since the positive temperature effect on larval fish growth is a well-known phenomenon (Elliot, 1982).

The value obtained in the current study at 22 °C corresponded closely to the value of  $0.80 \text{ mm}\cdot\text{d}^{-1}$  obtained by Fey et al. (2018) for

northern pike fed artificial feed under experimental conditions at 18 °C during the first 28 days of life. Wolska-Neja and Neja (2006) reported a similar value ( $0.81 \text{ mm}\cdot\text{d}^{-1}$ ) for pike during the first 27 days, but at lower temperatures of 13.5–18.5 °C. The faster growth reported in this publication despite the relatively low temperature might have resulted from feeding the larvae natural zooplankton. It seems, therefore, that this relatively high temperature of 22 °C is not above optimum for the growth of larval and early juvenile northern pike. Although (Szczepkowski, 2006, 2009) concluded that even a temperature of 28 °C is not above optimum for pike growth, this is not necessarily valid for larval life stages because his work was based on large juveniles. However, considering research on the ecology of early life stages of pike conducted under natural conditions, temperatures above 22 °C are very occasional,



**Fig. 7.** The relationship between otolith size (sagittae and lapilli) and standard length (SL) of larval northern pike *Esox lucius* during the first 28 days of life under controlled conditions. The data were divided into two growth rate (GR) groups: GR above mean (positive residuals of the best-fit regressions fitted to SL at age data) and GR below mean (negative residuals of the best-fit regressions fitted to SL at age data) (a and b); and into three temperature groups: 10, 15, and 22 °C (c and d). Insert graphs show the same data but for comparable SL range, and described with linear regressions. To obtain linearity for GLM and ANCOVA analysis the SL was Log-transformed for lapilli. The annotation for Y axis is for the insert graphs is the same as for the large graphs.

and there is a very low probability of obtaining otolith material from pike that experienced higher temperatures than those used in this research.

Interestingly, the highest temperature group was characterized in this study by GR increase among relatively older larvae and the highest SL at age variation. Except the temperature effect itself, it is highly likely that those differences were related also to the presence of cannibalism phenomenon (Folkvord and Otterå, 1993). As it was recently showed for larval and early juvenile pike within first month of life (Greszkiewicz and Fey, 2020), temperature has a significant positive influence not only on the time (i.e., age and size of larvae) of the cannibalism initiation but also on the cannibalism intensity. The more cannibalistic attacks occurs, the more the size of larvae within a given group/population will be diverse. At least to the point when only the most active cannibals are left. In such case opposite may be truth and the size range may be diminished.

#### 4.2. Temperature and somatic growth effect on otolith growth

Somatic growth in northern pike was followed closely in the current study by otolith growth both within each of the three temperatures and among them. Thus, the results obtained confirmed reports by many authors that otolith size (increment width) provides direct evidence of somatic growth (e.g., Mugiya and Oka, 1991; Maillet and Checkley, 1990; Paperno et al., 1997; Tonkin et al., 2008). Most recently, Do Souto et al. (2019b) reported for Argentine anchovy, *Engraulis anchoita*, larvae that both recent otolith growth and mean daily otolith growth provided valuable data on somatic growth differences among seasons within a year. Moreover, recent otolith growth performed as a somatic growth indicator better than the RNA/DNA index.

The confirmation of somatic and otolith growth proportionality in the present research was an important outcome in view of results obtained in some other studies which indicated that above a certain temperature otolith growth can continue to increase despite somatic growth stopping or decelerating significantly. This was noted in early life stages of species from distinct environments, for example, Arctic char,

**Table 3**

Results of linear regression analysis of otolith size (sagittae maximum diameter, SMD; lapilli maximum diameter, LMD) at standard length (SL) of larval pike during the first 28 days of life at three temperatures. The data were divided into two growth rate groups: GR below mean value (Low GR, negative residuals of SL at age regression) and GR above mean value (High GR, positive residuals of SL at age regression), and into three temperature groups: 10, 15, and 22 °C. Only data points representing the same range in each comparison were used.

Group	Linear regression	n	R <sup>2</sup>	P
<i>Sagittae</i>				
High GR	SMD = -144.1283 + 21.9569 SL	259	0.925	P < 0.001
Low GR	SMD = -183.6423 + 24.2507 SL	266	0.926	P < 0.001
<i>Lapilli</i>				
High GR	LMD = -297.1329 + 159.3559 Log SL	259	0.950	P < 0.001
Low GR	LMD = -352.5399 + 180.5296 Log SL	262	0.958	P < 0.001
<i>Sagittae</i>				
10 °C	SMD = -121.893 + 19.512 SL	147	0.833	P < 0.001
15 °C	SMD = -146.1839 + 21.7807 SL	119	0.919	P < 0.001
22 °C	SMD = -134.2877 + 22.1624 SL	90	0.864	P < 0.001
<i>Lapilli</i>				
10 °C	LMD = -24.6735 + 10.4642 Log SL	147	0.809	P < 0.001
15 °C	LMD = -25.2875 + 10.6832 Log SL	116	0.908	P < 0.001
22 °C	LMD = 6.1191 + 8.5919 Log SL	89	0.859	P < 0.001

**Table 4**

Results of GLM (unequal slopes model) and ANCOVA for the effect of somatic growth (GR) and temperature (Temp) (grouping variables) and standard length (SL) (covariate) on otolith maximum diameter (dependent variable) of larval pike during the first 28 days of life. The GR groups were: GR below mean value (negative residuals of SL at age regression) and GR above mean value (positive residuals of SL at age regression). The temperature groups were: 10, 15, and 22 °C.

	SS	df	MS	F	P
<i>GR effect for sagittae (GLM unequal slopes model)</i>					
Intercept	459876	1	459876	1312	P < 0.001
GR*SL	2270892	2	1135446	3241	P < 0.001
GR	6683	1	6683	19	P < 0.001
Error	182523	521	350		
<i>GR effect for lapilli (GLM unequal slopes model)</i>					
Intercept	233800	1	233800	3623	P < 0.001
GR*SL	477068	2	238534	3696	P < 0.001
GR	1700	1	1700	26	P < 0.01
Error	33361	517	64		
<i>Temperature effect for sagittae (ANCOVA)*</i>					
Intercept	81822	1	81822	436	P < 0.001
GR*SL	477283	1	477283	2546	P < 0.001
GR	38607	2	19303	103	P < 0.001
Error	65963	352	187		
<i>Temperature effect for lapilli (GLM unequal slopes model)</i>					
Intercept	42616	1	42616	859	P < 0.001
GR*SL	108235	3	36078	727	P < 0.001
GR	627	2	313	6	P < 0.01
Error	17160	346	49		

\* Because of no differences in slopes (interaction term insignificant), ANCOVA was used for intercepts comparison.

*Salvelinus alpinus* (Mosegaard et al., 1988), Norwegian spring-spawning herring, *Clupea harengus* (Folkvord et al., 2004), Baltic herring (Fey, 2001, 2005, 2006), smelt (Fey, 2006), Atlantic cod, *Gadus morhua* (Neat et al., 2008), glass eel, *Anguilla japonica* (Fukuda et al., 2009), and Atlantic menhaden (*Brevoortia tyrannus*) (Fey and Hare, 2012). It seems that the key factor leading to the occurrence of this phenomenon of uncoupling in somatic and otolith growth is temperatures exceeding optimal values for growth while metabolic rates continue to increase

(Wright, 1991; Wright et al., 1990), so that otolith and somatic growth are coupled only at optimum temperatures for somatic growth (Mosegaard et al., 1988; Hoff and Fuiman, 1993) (Larson and Berglund, 1998) Fey, 2001, 2005, 2006). Thus, the occurrence of this phenomenon in a given environment will be species-specific in the same way that the optimal temperature for growth is species specific (for a review of physiology and otolith characteristics see Grønkvær, 2016). For example, Fey (2006) showed for two pelagic species occurring at the same time in the same environment that otolith growth was more dependent on somatic growth in smelt and more dependent on temperature in herring. In the current work on larval and early juvenile northern pike, the highest temperature (22 °C), despite it being at the high end of values encountered by pike in natural conditions, was within the optimum for growth, and otolith growth dependence on somatic growth was not disrupted.

#### 4.3. Temperature and somatic growth effect on the otolith size-fish size relationship

The relationship between the sizes of otoliths and fish size was strong, but with a statistically significant temperature and somatic growth effects. Considering temperature influence, only for sagittae it was possible to describe the observed pattern unambiguous way as having larger otoliths by larvae of a given SL in higher temperature. For lapilli the temperature effect was difficult to assess due to its unclear pattern. The positive temperature influence on the otolith size-fish size relationship has been already reported for herring larvae that, as in the present research, experienced the highest temperature limits for this species (Fey, 2001, 2005, 2006). One may expect that the presence and magnitude of this phenomenon is related not only to temperature level but also to species-specific otolith growth rate. For sagittae of pike larvae that rate is very high (Fey et al., 2018), as it is also the case for mentioned above herring larvae (Fey, 2006).

Considering somatic growth effect on the otolith size-fish size relationship, when the data were split into two groups, GR above and below mean, the GR effect was statistically significant for both sagittae and lapilli. However, it was not possible to describe those patterns definite way because the magnitude of the differences was SL-dependent. The uncoupling in the otolith size-fish size relationship was reported before by other authors, for example in Arctic char (Mosegaard et al., 1988), striped bass, *Morone saxatilis* (Secor and Dean, 1989), herring (Fey, 2001; 2006), and smelt (Fey, 2006). The mechanism of this phenomenon is that slower-growing specimens of the same size are older and more increments are deposited in their otoliths. Consequently, their otoliths are larger at a given SL. Since the occurrence and magnitude of the uncoupling in the present research was fish size-dependent, it can't be concluded for pike that slower growing specimens have larger otoliths, and it is difficult to point towards possible mechanism of this phenomenon. Although the GR and temperature effects on otolith size-fish size relationship were statistically significant, it should be noted that the magnitude of this effects was relatively low - as it can be assessed by visual inspection of the graphical presentation of those data.

#### 4.4. Consequences for growth rate back-calculation

The results of the present work confirm that otolith and body size uncoupling should be considered as a potential source of error in growth back-calculation. Klumb et al. (2011) report this, for example, in their paper on validating three back-calculation models. More recently Morrison et al. (2019) reported the uncoupling of otolith and somatic growth during anadromous migrations in northern salmonids. Because the largest bias can be expected in proportional-based models that do not account for the growth effect (Vigliola and Meekan, 2009), several back-calculation models that account for the uncoupling in somatic and otolith growth have been proposed (Sirois et al., 1998; Vigliola et al., 2000; Morita and Matsuishi, 2001; Finstad, 2003; Ashworth et al., 2017). Their performance is, however, also limited in specific

circumstances. As Morrison et al. (2019) point out, these models provide accurate results only if no factors other than age or growth affect uncoupling in somatic and otolith growth. One such factor could be ontogenetic shifts during life history events like migrations (Morrison et al., 2019). To account for this factor, Morrison et al. (2019) developed a breakpoint method of growth back-calculation. Similarly, Hobbs et al. (2007) considered variability in the otolith size-fish size relationship among different developmental stages in their stage-specific biological intercept back-calculation model. Thus, obtaining accurate results from back-calculation methods is challenging and requires not only accounting for the growth rate of larvae but also their developmental stage to correctly describe the otolith size-fish size relationship. As shown in the present research, the experienced temperature should be considered as well.

Although pike is a species that inhabits waters of distinct salinities, no information about the effect of salinity on otolith-somatic growth uncoupling is available in the literature. It cannot be excluded that the salinity factor might introduce additional complexity into the relationships studied in this research.

#### 4.5. Conclusions

Northern pike otoliths (sagitta and lapillus), the growth of which reflects somatic growth closely, provided a reliable source of information on the early life history of this species, and they can be used with methods based on increment width measurements, like increment width trajectory analysis and marginal increment width analysis, in a wide spectrum of temperatures occurring in natural conditions (10–22 °C). However, one must be cautious with growth back-calculation because of the temperature and somatic growth effects on the fish size-otolith size relationship, which was statistically significant despite of its relatively low magnitude when assessed visually. Thus, the use of proportional back-calculation methods must be substituted with more complex models.

#### CRedit authorship contribution statement

**Dariusz P. Fey:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Martyna Greszkiewicz:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization.

#### Declaration of Competing Interest

The authors report no declarations of interest.

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## OŚWIADCZENIE

Współautorzy artykułu pt.: "Effects of temperature on somatic growth, otolith growth, and uncoupling in the otolith to fish size relationship of larval northern pike, *Esox lucius* L" opublikowanego w czasopiśmie naukowym Fisheries Research, <https://doi.org/10.1016/j.fishres.2020.105843>, zgodnie oświadczają i podpisani poświadczają, że wkład poszczególnych osób w powstanie publikacji i wynikający z tego procentowy udział był następujący:

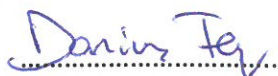
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- udział w pisaniu tekstu i przygotowaniu manuskryptu
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Dariusz Fey



Martyna Greszkiewicz

**Artykuł III** - Greszkiewicz Martyna, Fey Dariusz P. (2020). Positive temperature effects on the initiation and intensity of cannibalistic behaviour of larval pike, *Esox lucius* L. Is cannibalism reflected in otolith fluctuating asymmetry? *Hydrobiologia* 847, 3139–3152. <https://doi.org/10.1007/s10750-020-04328-5>





# Positive temperature effects on the initiation and intensity of cannibalistic behaviour of larval pike, *Esox lucius* L. Is cannibalism reflected in otolith fluctuating asymmetry?

Martyna Greszkiewicz · Dariusz P. Fey

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**Abstract** Cannibalism can be a significant factor limiting the survival of pike, *Esox lucius*, early life stages in both natural and aquaculture conditions. In the present research, type I cannibalism was studied in pike larvae (size range: 9–32.5 mm standard length, SL) in controlled conditions for 45 days post-hatching (dph). For this purpose, the occurrence of cannibalistic pairs, i.e. prey caught by a cannibal inside the cannibal's mouth, was monitored for 15 min periods by two observers six times daily. Cannibalism was not observed in fish that still had yolk-sacs, but it did occur as soon as the yolk-sac was absorbed on 5 dph at a body size of 13 mm at 22 °C and 10 dph at a body size of 13.4 mm at 15 °C. The ratio of prey to predator size was on average 87%, but the smallest observed size difference was only 1.3%. The intensity of cannibalistic attacks depended on water temperature with more intense cannibalism at 22 °C than at 15 °C, and no cannibalism at 10 °C. Fluctuating asymmetry (FA) in all three sagittal otolith size parameters (area, width, and length) was higher in prey than in cannibals, the differences were not statistically significant.

**Keywords** Cannibalistic behaviour · Fish larvae · Mortality · Bilateral instability

## Introduction

The phenomenon of cannibalism is observed in many animal groups, *inter alia*, in protozoa, rotifers, snails, copepods, mites, insects, spiders, fish, amphibians, birds, and mammals (Fox, 1975). Increasing evidence indicates that cannibalism is not only common but that it is important to the ecology of many species. Fox (1975) concluded that cannibalism is a natural behaviour in 147 species, while Polis (1981) cites reports on approximately 1300 species. Smith & Reay (1991) define cannibalism more precisely as an act of killing and consuming wholly or in large part an individual belonging to the same species regardless of developmental stage. Similarly, Garcia & Zaniboni-Filho (2006) defines cannibalism as a special type of predation that depends on killing individuals of the same species for partial or total consumption.

Among fish, the phenomenon of cannibalism has been identified unequivocally in the literature in 36 of 410 teleost fish families (Nelson, 1984). It is likely, however, that cannibalism in fish is more widespread than a literature review can suggest (Dominey & Blumer, 1984) since the phenomenon has been described in the literature mainly in species that are

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of interest to humans as resources, are used as models in behavioural studies, or are popular species in fishkeeping (Smith & Reay, 1991). Pereira et al. (2017), who reviewed more than 1000 articles on fish cannibalism, conclude that this phenomenon among marine fishes occurs most frequently among Gadidae (106 records, Atlantic cod, *Gadus morhua* dominant), Gobiidae (54 records, common goby, *Pomatoschistus microps*, dominant), and Merlucciidae (29 records, genus *Merluccius* dominant), while among freshwater fishes it occurs among Percidae (140 records, European perch, *Perca fluviatilis*, and yellow perch, *P. flavescens*, dominant), Salmonidae (75 records, Arctic char, *Salvelinus alpinus*, dominant), and Esocidae (54 records, Northern pike, *Esox lucius*, dominant).

Although cannibalism occurs in both early developmental stages and adult fish, most instances of cannibalistic behaviour refer to the consumption of larvae or juvenile individuals (Pereira et al., 2017). This is true for the pike analysed in the current study in which cannibalism is particularly widespread in the first year of life (Craig, 1996). Feeding habits in young pike change as they grow and three feeding stages occur during development (Hunt & Carbine, 1951). This feeding sequence progresses with pike growth from lower crustaceans to insects, and then to fish. It is supplemented, even at an early developmental stage (15–20 mm), by attacks on larval fish, including cannibalistic attacks (Hunt & Carbine, 1951; Morrow et al., 1997; Mamcarz et al., 1998; Žiliukienė & Žiliukas, 2006).

Cannibalism is divided into seven classes according to the developmental stage of the prey, the genetic relationship between the cannibal and its prey, and the age relationship between them (Smith & Reay, 1991). According to the classification by Smith & Reay (1991), pike belongs to the class of “sibling intracohort cannibalism of post-hatching stages.” This class is characterized by sibling cannibalism or individuals belonging to the same age class, which is typical of piscivorous species in larval and juvenile early life stages (Naumowicz et al., 2017). Bearing in mind the methodology used in the present research and size range of analysed fish, type I cannibalism is considered. This occurs when the size of the prey is large relative to that of the predator, it can be ingested tail first (or less frequently head first) and consumed (Cuff, 1980); this is in contrast to type II cannibalism, which is characterized by the cannibal immediately and

completely ingesting the prey (Baras & Jobling, 2002).

The ability to eat siblings in early life stages before offspring disperse, which occurs in pike, is facilitated by the pike’s large gape and well-developed dentition shortly after hatching (Giles et al., 1986). Cannibalistic larvae usually have augmented dentition, large gape size, and increased jaw musculature (Folkvord, 1997). In both adult and early life stages of pike, the long build of the body and its articulated jaw and backward-facing hinged teeth prevent prey from escaping, and the combination of the propellant force of the anal, caudal, and dorsal fins helps to obtain high acceleration when attacking prey (Craig, 1996; Skov & Nilsson, 2018). Additionally, the underslung pairs of pectoral and pelvic fins enable precise manoeuvring to sight prey stereoscopically down paired snout grooves (Craig, 1996).

In the natural environment, cannibalism is a key mechanism for regulating pike abundance that has significant implications for pike year class recruitment (Craig & Kipling, 1983) and also for the structure of given populations (Skov & Nilsson, 2018). It is especially important for over-exploited populations, which can be found not only in freshwater systems but also in many coastal areas of the Baltic Sea (Nilsson et al., 2014; Larsson et al., 2015; Skov & Nilsson, 2018). Although the main reasons for population declines are over-exploration and the disappearance of suitable spawning grounds and low recruitment (Skov & Nilsson, 2018), other factors related to human activities in coastal areas (e.g. wind farms and underwater cables) should also be considered (Fey et al., 2019). When there is a lack of recruitment from natural spawning, local populations are often supported by stocking with pike larvae or fry obtained from recirculating aquaculture systems (RAS), which have been found to be very efficient in producing pike stocking material (Szczepkowski et al., 2012). Unfortunately, cannibalism among pike early life stages plays a significant role not only in natural but also in aquaculture conditions because it can cause total losses that exceed 90% of total mortality (Szczepkowski, 2009). The significance and potential consequences of the occurrence of cannibalism during larval and juvenile stages mean it is extraordinarily important to acquire an understanding of this phenomenon.

There are several methods for determining the condition and fitness of fish specimens, including

methods that evaluate Fulton's condition factor (Jin et al., 2015), morphology and histology (Theilacker, 1986), lipid and protein composition (Håkanson, 1989; McCormick & Molony, 1993), RNA to DNA ratios (Clemmesen, 1988; Folkvord et al., 1996; Peck et al., 2015), and otolith microstructure (Campana & Jones, 1992). Alternatively, fitness is reflected in developmental instability (Zakharov, 1992). Developmental instability can then be reflected in fluctuating asymmetry (Palmer, 1994), which is defined as a random deviation in a perfect bilateral system (Palmer, 1994). Assuming environmental stress causes developmental instabilities, high fluctuating asymmetry can indicate the lower condition of larvae that experienced unfavourable environments. The link between fish condition and fluctuating asymmetry has been studied in both adult and larval fish, and a number of measurements have been analysed, including otolith size and shape (Escós et al., 1995; Somarakis et al., 1997a, b; Fey et al., 2020). However, the results obtained in different studies are inconsistent. Presumably, whether fish larvae belong to cannibal or prey groups within a cannibalistic population depends on differences in both growth rates and fish condition. The question posed in the present study is whether there are also differences in developmental instability that are reflected in fluctuating asymmetry in otolith size between groups of cannibals and prey. Although otolith growth and daily otolith increment deposition have recently been analysed for larval and early juvenile northern pike (Fey et al., 2018), no information on otolith asymmetry has been published to date.

The aim of the present study was to determine for larval pike (9–32.5 mm SL) held under controlled conditions if the water temperature (10, 15, 22 °C) had an effect on the age and size of predators during the first cannibalistic attacks, the intensity of the cannibalism phenomenon, and the proportion of prey to predator body size. The methodology used chosen to analyse type I cannibalism (the cannibal ingests its prey by the tail or head first). Additionally, whether belonging to predator or prey groups was related to the presence of higher or lower organism bilateral instability was also verified by analysing otolith size fluctuating asymmetry.

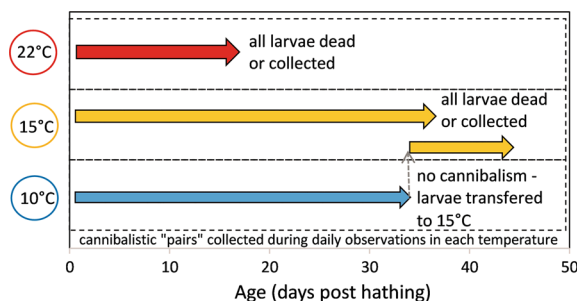
## Materials and methods

### Experimental conditions

The experiments were performed in nine freshwater tanks with volumes of 55 L each combined into three RAS providing three temperatures (mean  $\pm$  SD): 10  $\pm$  0.6 °C, 15  $\pm$  0.4 °C, 22  $\pm$  0.5 °C, with three replicates of each temperature (3 temperatures  $\times$  3 replicates). Each of the systems was equipped with UV sterilization and a filtering-deposit container with a volume of 70 L providing both mechanical and biological filtration. The volume of biological filtration was 20 L. In each of the tanks a green plastic net (mesh size 0.5 cm) formed into a roller (20 cm in length  $\times$  8 cm in diameter) was used as a substitute for bottom substrate. The colour of the tank bottom was grey, and the glass walls were not covered. The tanks were located in a completely dark room in which light conditions ( $\sim$  100 Lux on the water surface, 12 h light/12 h dark, with light on from 8.00 to 20.00) were regulated automatically. Water temperature was measured three times daily with electronic temperature readers. The oxygen level during the experiment was measured daily and was 7.8  $\pm$  0.9 mg l<sup>-1</sup> in all tanks (mean  $\pm$  SD) throughout the experiment.

The rationale for choosing the particular temperatures was to cover the wide range of temperatures that larvae encounter in natural conditions depending on the type of water body and depth. Temperatures typically range between 10 and 15 °C, but they can exceed 22 °C in shallow flooded fields where the water depth is only several centimetres. Two temperatures (15 °C and 22 °C) correspond to conditions that can occur in aquaculture systems.

The total time of experiment was 45 days, but this differed depending on the temperature: 17 days at 22 °C (no fish left beyond this point), 37 days at 15 °C (no fish left beyond this point), and 34 days at 10 °C (no cannibalism occurred). On day 34, the fish from the 10 °C treatment were transferred to the 15 °C treatment for 11 days (group 10–15 °C). The rationale behind the transfer from 10 to 15 °C was to test if cannibalism was triggered at the higher temperature and after how many days (see results for the information about no cannibalism at 10 °C). The scheme of the experimental setup is presented in Fig. 1.



**Fig. 1** Scheme of the experimental setup. The transfer of larvae from 10 to 15 °C on day 34 was a consequence of no cannibalistic attacks at 10 °C

### Fish larvae rearing

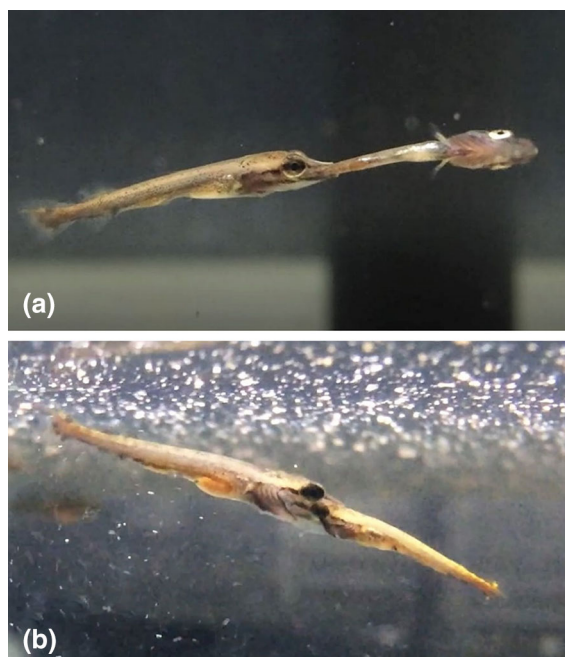
Eyed-egg pike embryo were transported to the laboratory from a commercial hatchery, and 3000 eggs obtained from one female were placed in separate bags for transportation. The number of eggs was estimated by the volumetric method using a glass cylinder with a calibration scale (Szczerbowski, 2008). Mass hatching occurred during the first hour of transportation, and the hatched larvae were placed in tanks (one bag in one tank). The unhatched eggs left in the bags were counted to estimate the number of larvae in each tank. Hatching success was within a range of 92–95% in all of the transportation bags. The temperature was set to 10 °C in each tank at the beginning of the experiment. Thereafter, the temperature was increased to 15 °C within 24 h in one of the systems and to 22 °C within 48 h in the other one, and it was constant thereafter. Following yolk-sac absorption, feeding was conducted ad libitum every 1.5 h during periods with light with specialist fodder, as follows: ArtEX 2 (0.15 mm) (Aller Aqua) for the first 4 days after yolk-sac absorption; Perla Larva Proactive 4.0 (0.3–0.5 mm) (Skretting AS) from day 3 after yolk-sac absorption. Dead fish, unconsumed fodder, and excrement were cleaned from the tank bottoms every morning. The number of dead fish in each tank was counted to calculate the current density of larvae in each tank as well as the cannibalism intensity index (see data analysis section).

### Cannibalism analysis

During each feeding (i.e. six times daily, every 1.5 h during the light period), the occurrence of

cannibalistic pairs, i.e. prey caught by a cannibal inside its mouth (Fig. 2), was monitored in all tanks simultaneously for 15 min periods by two observers. Because only cases when prey was inside mouth of the cannibal were counted as cannibalistic attacks, this approach can be considered to be the analysis of successful cannibalism. Each pair was collected, and after placing it in 800 mg l<sup>-1</sup> MS-222 anaesthetic fluid (ACROS Organics™, Belgium), it was transferred into an individual vial (one pair per vial) of 96% alcohol to preserve the material for otolith asymmetry analysis. Although the approach described did not provide information on the absolute number of cannibalistic attacks, it permitted calculating the relative type I cannibalism intensity index that is comparable among the three temperatures. A total of 71 cannibalistic pairs was collected.

The alcohol in each vial was changed after two weeks since it has been demonstrated that alcohol storage can damage otoliths and affect the results of otolith size measurements (Fey, 2018), which would



**Fig. 2** Picture of a cannibalistic pair of pike (*Esox lucius*) representing type I cannibalism (without immediate ingestion). The cannibal holds the prey, which is not much smaller, by the tail (a). Once the difference in size between cannibals and prey increased, the attacks from the side and head were also observed (b). Only instances in which the prey's body was inside the cannibal's mouth were considered to be cannibalistic attacks in this study. All "connected" pairs were collected and analysed

influence the results of otolith size asymmetry analysis performed for the current study. Cannibal and prey body sizes (standard length, SL) were measured with an electronic calliper to the nearest 0.1 mm a few weeks after collection. For the purpose of background description (i.e. larvae size, both prey and cannibals, in a given tank in a given temperature), 10 larvae were collected every three days and placed first in the anaesthetic fluid and then in alcohol. The SL of these larvae were measured to the nearest 0.1 mm within a few weeks. The size of the preserved specimens was corrected for alcohol shrinkage, and unpreserved size was calculated with a correction factor (+0.7 mm) (Greszkiewicz & Fey, 2018). The size range of the larvae collected from cannibalistic pairs was between 11.8 and 32.5 mm SL.

#### Otolith fluctuating asymmetry analysis

Otoliths (left and right sagitta) were extracted from each larva and placed in DePeX mounting medium (Electron Microscopy Sciences, Fort Washington, PA) on microscope slides. The size of the otoliths was measured with an image analysis system (Image-Pro Premier, Media Cybernetics, Inc., Rockville, MD) under an Eclipse 80i transmitted light microscope (Nikon Corp., Tokyo, Japan).

The fluctuating asymmetry of otoliths (left otolith—L, and right otolith—R) was analysed according to methods described by Fey & Hare (2008). First, it was verified that the (L–R) was not different from zero (one-sample *t* test to 0) and that the distribution of differences was normally distributed (Shapiro–Wilk *W*-test) and free from antisymmetric (bimodality or platykurtosis) and directional symmetry (skew). Linear regression analysis was then employed to test if the absolute difference between the left and right otolith size (|L – R|) depended on trait size (i.e. otolith size). The largest measurement (left or right otolith) was used as the trait size since Sullivan et al. (1993) demonstrated that using the mean value as trait size can introduce a spurious correlation and should be avoided. The analysis was performed for cannibals and prey separately. Since the |L – R| was not dependent on trait size, the mean |L – R| was used as the fluctuating asymmetry index (FA). The comparison was based on the  $n = 71$  cannibals and  $n = 71$  prey sample size.

#### Data analysis

The time (dph) of the first cannibalistic attack and the size of the cannibals in the attacks were compared among the temperatures. How cannibalism intensity changed over time (i.e. with changing fish density in tanks and fish size) and what the effect of temperature was on the phenomenon were analysed with the cannibalism intensity index CI (%) that was calculated for each sampling day:

$$CI = N_{\text{canib}}/N_{\text{all}} \cdot 100$$

where  $N_{\text{canib}}$  is number of cannibalistic pairs collected on a given day in a given tank and  $N_{\text{all}}$  is number of all larvae in the tank.

The number of larvae in a given tank on a given day was estimated by subtracting the number of dead fish found during morning cleaning on that day and during previous days from the number of fish in that tank at the beginning of the experiment (i.e. number of eggs in the transportation bag minus unhatched eggs).

The CI was plotted against SL (mm) and larvae density ( $n \cdot L^{-1}$ ) separately for the three temperature groups. The growth rate of the cannibals and the prey was compared with slope comparison (ANCOVA) of linear functions fitted to SL at age data. The differences in the FA between cannibals and prey were evaluated using the *t* test. All those analyses were conducted after data were pooled among the three subsample tanks for each temperature to obtain a sufficient number of observations for this calculation. Still, it was beneficial to have subsamples from three tanks as the CI index calculations were performed for the specific conditions (density and larval size) the larvae encountered in each of the tanks. Statistica, version 12.0 (TIBCO Software, Inc., Palo Alto, CA) software was used for data analysis. Differences were considered statistically significant at  $P < 0.05$  ( $\alpha = 0.05$ ).

## Results

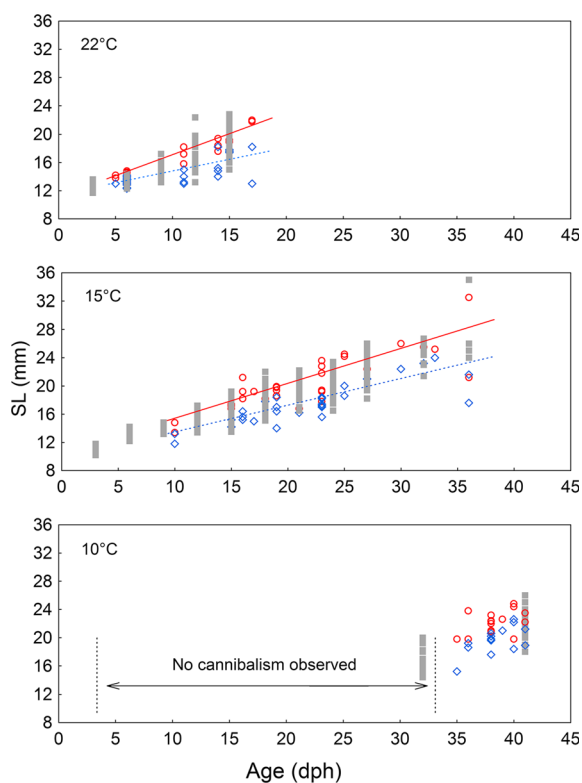
#### First cannibalistic attack—cannibal size and age

In total, 71 cannibal-prey pairs representing larva size ranges from 11.8 to 32.5 mm SL were collected during the experiment. Thus, successful attacks were evaluated without considering unsuccessful attacks or bites



only. The first successful cannibalistic attack was observed 5 dph at 22 °C, 10 dph at 15 °C, and no attacks were observed at 10 °C (Fig. 3). Cannibal size at the first attack was 13.0 mm at 22 °C (Fig. 3a) and 13.4 mm at 15 °C (Fig. 3b). When the fish were transferred from 10 to 15 °C (34 dph, size range 14.4–19.4 mm), the cannibalistic attacks started after 5 days at a cannibal size of 19.8 mm (Fig. 3c). Fish from the full-size range present in the tanks at a given time participated in the cannibalistic phenomenon with cannibals representing approximately the upper half of the size range of all the larvae in the tanks and the prey representing the lower half of the size range (Fig. 3).

The first attacks at 15 and 22 °C were from the tail only (Fig. 2a). At a cannibal size of approximately 17 mm, attacks from the side and head (Fig. 2b) were also noted and they represented 10% (side) and 14%



**Fig. 3** Standard length (SL, mm) of larval pike, *Esox lucius*, cannibals (circle and solid line), prey (diamond and dashed line), and other specimens present in the tanks (filled square) on individual days after hatching at three different temperatures: 22 °C (A), 15 °C (B), 10 °C (C). Each point represents an individual fish. The data were pooled for three tanks at each temperature

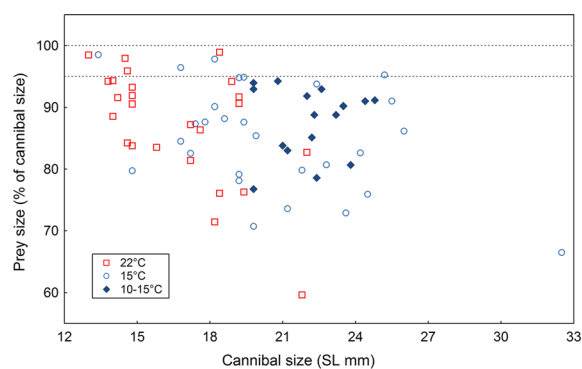
(head) of all the attacks among larvae from the 17–32 mm size range.

#### Prey to cannibal size ratio

The prey size to cannibal size proportion ranged, on average, from 95% for 13 mm fish to 80% for 27 mm fish, with a mean for all specimens of 87% (Fig. 4). The smallest differences in size between the prey and cannibals (approximately 0.2 mm) occurred in the cannibal size range from 13.4 (difference = 1.5%) to 18.4 mm (difference = 1.1%). There was no temperature effect on the analysed proportion (Fig. 4).

#### Growth rate of cannibals and prey

The growth rates of cannibals and prey was estimated from the slopes of regression lines fitted to SL at age data. The growth rate of larvae at 22 °C was 0.57 mm·day<sup>-1</sup> for cannibals and 0.32 mm·day<sup>-1</sup> for prey (Fig. 3a). At 15 °C, the growth rate of cannibals was 0.55 and of prey it was 0.43 mm·day<sup>-1</sup> (Fig. 3b). The differences between the growth rates of cannibals and prey were statistically significant for both 15 and 22 °C (ANCOVA, slope:  $P < 0.05$ ). Since the slopes were different, no comparison was made for the elevations of the regression lines. In the 10–15 °C group, growth was not calculated because of the narrow size range of the larvae. However, the cannibals were clearly larger at age (i.e. at dph) than



**Fig. 4** Percentage values of the proportion of prey size to cannibal size of larval pike, *Esox lucius*, at three different temperatures: 22 °C (squares), 15 °C (circles), 10–15 °C (diamonds; fish transferred from 10 to 15 °C after 34 days of the experiment). The range of the highest similarities (range of 95–100%) in sizes between cannibals and prey is marked with a dashed line

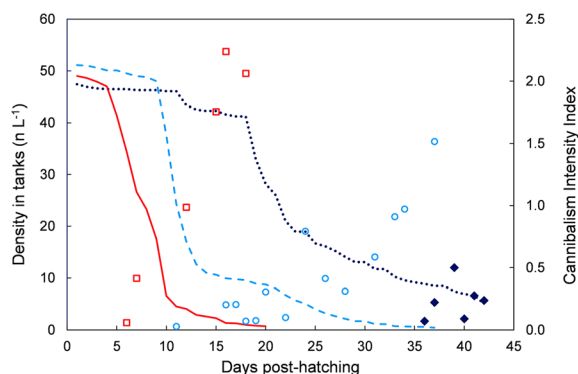
the prey (Fig. 3c), and the difference was statistically significant (t test,  $P < 0.01$ ). This difference indicated that the growth rate of cannibals was faster than that of the prey.

#### Temperature effect on cannibalism intensity

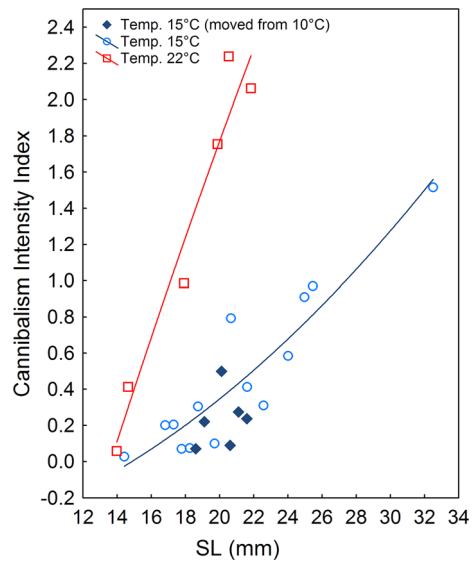
Cannibalism intensity increased with time at each temperature (Fig. 5), and it corresponded to decreasing larval density in the tanks (Fig. 5) and to increasing fish size (Fig. 3). To account for these two factors—density and fish size—the effect of temperature on CI was analysed by plotting CI against SL (Fig. 6) and against fish density in the tanks (Fig. 7). In both comparisons, cannibalism intensity was higher at 22 °C compared to the 15 °C and 10–15 °C groups. Cannibalism intensity in the 15 °C groups was the same as in the 10–15 °C group. These differences were statistically significant (ANCOVA for log-transformed data, intercept  $P < 0.05$ ).

#### Cannibalism and otolith fluctuating asymmetry

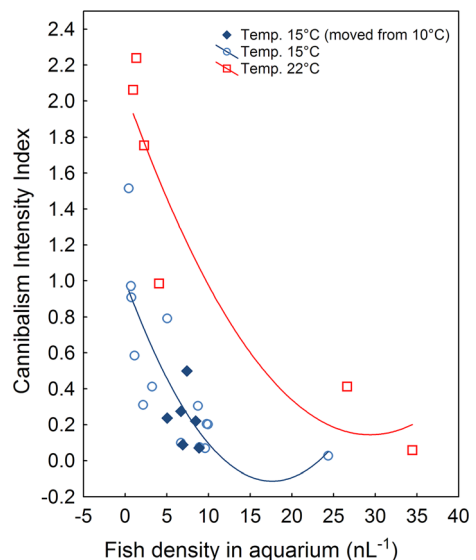
The FA of otolith size (area, length, and width of sagitta) was compared between prey ( $n = 71$ ) and cannibals ( $n = 71$ ) (Table 1, Fig. 8). Although the FA for all the otolith size variables was higher for prey compared to cannibals, the differences were not statistically significant (paired t test,  $P > 0.05$ ).



**Fig. 5** Comparison of changes in the timing of cannibalism intensity (Cannibalism Intensity Index) and density of larvae in tanks for pike, *Esox lucius*, at three different temperatures: 22 °C (square and solid line), 15 °C (circle and dashed line), 10–15 °C (fish transferred from 10 to 15 °C after 34 days of the experiment) (diamonds, dotted line)



**Fig. 6** Cannibalism intensity (Cannibalism Intensity Index) depending on individual standard length for larval pike, *Esox lucius*, at three different temperatures: 22 °C (square and solid line), 15 °C (circle and solid line), 10–15 °C (fish transferred from 10 to 15 °C after 34 days of the experiment) (diamonds, no line was fitted)

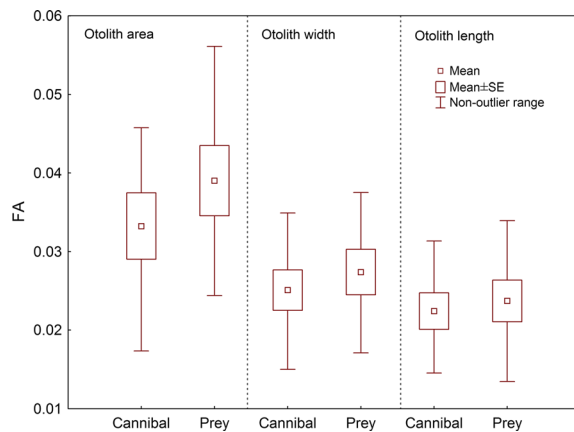


**Fig. 7** Cannibalism intensity (Cannibalism Intensity Index) depending on fish density in tanks for larval pike, *Esox lucius*, at three different temperatures: 22 °C (square and solid line), 15 °C (circle and solid line), 10–15 °C (fish transferred from 10 to 15 °C after 34 days of the experiment) (diamonds, no line was fitted)



**Table 1** Fluctuating asymmetry indexes (FA) (Mean  $\pm$  SD) of sagittal otolith size calculated for cannibals ( $n = 71$ ) and prey ( $n = 71$ ) of larval pike, *Esox lucius*, kept under experimental conditions for 45 days

	FA (Mean $\pm$ SD)		
	Otolith area	Otolith length	Otolith width
Cannibals	0.036 $\pm$ 0.002	0.022 $\pm$ 0.002	0.025 $\pm$ 0.002
Prey	0.039 $\pm$ 0.002	0.024 $\pm$ 0.002	0.027 $\pm$ 0.002



**Fig. 8** Comparison (Mean, SD, and non-outlier range) of fluctuating asymmetry (FA) for otolith area, otolith width, and otolith length for larval pike, *Esox lucius*, cannibals ( $n = 71$ ) and prey ( $n = 71$ ). The differences between cannibals and prey were not statistically significant (t test,  $P > 0.05$ )

## Discussion

The results of the current study confirmed that cannibalism in pike is a common phenomenon, and it could possibly be a significant factor limiting survival in early developmental stages of the species. However, the cannibalism intensity values obtained in the present research, which were estimated during a few observations daily, were relative only. They were comparable among temperatures, but they did not provide an estimation of the absolute value of total mortality from cannibalism (for example during a 24 h period). This is especially so because total mortality from cannibalism resulted not only from the consumption of whole larvae, but also from death after bites even if the larvae attacked were not consumed (Szczepkowski, 2009). If we recalculate the mean daily mortality of 0.6% obtained in the present

research during 90 min/day observations into a 24 h period, a value of approximately 10% daily is obtained. This does not include deaths resulting from unsuccessful attacks. However, we believe that such a calculation is too speculative to include it in the results section.

## First cannibalistic attack—cannibal size and age

As the results of the present study show, pike cannibalism occurred as early as in the first days of life. The basic pike feeding pattern described by Hunt & Carbine (1951) can be divided into three stages. The first stage is from the moment pike begin exogenous feeding after yolk-sac resorption until they reach a length of about 25 mm when they feed mainly on lower crustaceans. In the second stage, which is during the period of growth from 26 to 50 mm in length, they feed mainly on immature aquatic insects. In the third stage, when pike are longer than 50 mm, the typical diet is composed almost exclusively of fish and other vertebrates. According to encyclopaedic information (Grabowska & Grabowski, 2013), cannibalism in this typical pattern only appears from a size of approximately 4 cm. Similarly, according to Wright & Giles (1987), pike larger than 30 mm feed exclusively on invertebrates. However, it is plausible that the exact timing (i.e. size and age) of the initiation of cannibalism depends on environmental conditions like temperature and feeding conditions.

During our study conducted under experimental conditions, the smallest cannibals observed were 13.0 mm (22 °C) or 13.4 (15 °C). However, no cannibalistic attacks occurred at 10 °C. These values are lower than those presented by other researchers to date regarding analyses of material obtained from natural conditions and during experiments. The occurrence of early cannibalistic attacks in the natural environment is confirmed, among others, by Žiliukienė & Žiliukas (2006), who report for pike that the smallest cannibalistic individuals were only 16 mm (mean water temperature of 15.7 °C). In the analyses conducted by Hunt & Carbine (1951), the size of the smallest cannibals was 21 mm, while Morrow et al. (1997) report a size of 25 mm. Giles et al. (1986) report that the size of the smallest cannibals under experimental conditions at a temperature of 12 °C was 30.3 mm. Wright & Giles (1987) report that cannibalistic attacks among pike cultured in ponds (no

temperature information was provided) occurred at a size of 49 mm. Kucharczyk et al. (1997) observed attacks committed by smaller sized fish of approximately 18–24 mm during experiments conducted at 20 °C.

In reference to the timing, or larval age, of the occurrence of cannibalism, the first cannibalistic attack at the highest temperature analysed (22 °C) was observed as early as 5 dph, while at the temperature of 15 °C, the first attack occurred 10 dph. At the lowest temperature (10 °C) no cannibalistic attacks were noted for 31 days (following which the larvae were transferred to the temperature of 15 °C). Kucharczyk et al. (1997) analysed the phenomenon of cannibalism in young pike stages in experimental conditions at a temperature of 20 °C, and the first cannibalistic attacks, similarly to those in the current study at a similar temperature, occurred at 5–7 dph. However, Giles et al. (1986) noted the first cannibalistic fry (at a size of 30.3 mm) on 32 dph, but since the study was conducted at a lower temperature (12 °C), this could have been a factor limiting the phenomenon of cannibalism in smaller specimens that was similar to that in our study at 10 °C. Other studies report on experiments in which the phenomenon of cannibalism did not occur despite high temperatures (18–24 °C) (Wolnicki & Górny, 1997). It is possible that at some point, too high temperature could cause stress that inhibits cannibalistic behaviour, and the results by Wolnicki & Górny (1997) from 24 °C could confirm this hypothesis. Our results, however, indicate that if such a stressful level of high temperature exists, it occurs above 22 °C.

The timing (i.e. size and age of larvae or juveniles) of the first cannibalistic attacks depends of course not only on temperature but also on larval density and feeding conditions in tanks or the environment. These differed in the studies cited above. However, even if their results are not fully comparable, they provide some estimation of the timing of the first cannibalistic attack in different conditions, including different temperatures. The early onset of cannibalistic behaviour in our study could have resulted from feeding conditions. Even if the larvae were fed *ad libitum*, the commercial feed might not have been as efficient as live food (zooplankton, larvae of other fish species) in the natural environment. On the other hand, it could happen frequently that the availability of food in natural conditions is insufficient, and the feeding

conditions could be similar to ours or worse. Thus, we assumed that the feeding conditions during the experiment were moderately good and were somewhere between very good and poor feeding conditions in nature.

Another factor affecting when cannibalistic behaviour begins is yolk-sac absorption, which can occur at various times depending on the temperature in which the larval pike are held. Fey et al. (2019) report that yolk-sac resorption in larval pike occurred 6 dph at 12 °C, while Demirci et al. (2017) report it occurring 10 dph at a temperature of 10 °C. In the current study, full yolk-sac resorption occurred 4 dph at a temperature of 22 °C, 6 dph at 15 °C, and 9 dph at 10 °C. The timing of yolk-sac resorption corresponded to the appearance of cannibalistic attacks, which were noted from 1 (at 22 °C) to 4 (at 15 °C) days after absorption. At 10 °C, no cannibalistic attacks were observed for the entire observation period, i.e. 25 days after yolk-sac resorption.

Thus, it can be concluded that factors facilitating the occurrence of cannibalism among larval pike was yolk-sac resorption and growth to a size of approximately 13 mm. This state can be obtained within 4 to 6 dph, depending on temperatures from 22 to 15 °C, respectively, which indicates a significant positive metabolism effect on the two factors, i.e. time to yolk-sac absorption and reaching the size of 13 mm. The impact of temperature is not restricted only to limiting the time until yolk-sac absorption and the larvae obtaining a given size. In the present study at a temperature of approximately 10 °C no attacks were noted even after yolk-sac resorption or when the larvae had achieved sizes considerably larger than 13 mm (up to 18 mm). This indicated that temperature affected the physiological state of the fish (Dabrowski, 1986). Clearly, metabolism and larval activity are reduced significantly and maintained at a minimal level below a certain temperature (Elliot, 1982).

#### Prey to cannibal size ratio

Nillson & Bronmark (2000) analysed the behaviour and morphology of pike and concluded that prey size is limited by its body depth and predator gape size. Body depth can be considered as both height and width since pike can attack from a lateral position. In the present work, the ratio of prey to predator ranged, on average, from 95% for 13 mm fish to 80% for 27 mm

fish, with a mean for all specimens of 87%. The smallest size difference between predator and prey was just 0.2 mm and was observed at both 22 °C and 15 °C. Such a small difference was evident at the beginning of the occurrence of this phenomenon (e.g. 5 dph, predator 13 mm—prey 12.8 mm) and during later periods (e.g. 14 dph, predator 18.4 mm—prey 18.2 mm). Žiliukienė & Žiliukas (2006) report that prey length was 90.9% of pike predators measuring 17.6 mm. However, Hunt & Carbine (1951) report this proportion was 70% for pike measuring 23 mm. Bry et al. (1992) demonstrated that for much larger pike held in semi-natural conditions the relative size of the prey in relation to the predator ranged from 35 to 85.4% and fluctuated linearly from approximately 75% at a cannibal total length (TL) of 60 mm to approximately 60% for cannibals measuring 200 mm TL. Clearly, the values of the size proportion between prey and predator decreased (larger differences) with predator growth because of increasing differences in size between the smallest and largest larvae in a given tank over time.

#### Type of cannibalism—direction of the attack

The present results confirm that the first attacks observed for pike within first few weeks after hatching represent type I cannibalism, in which the prey is only partially ingested from the tail or head before it is completely digested. Cannibalism type II, in which prey are completely ingested, appears with increasing size differences between cannibals and prey (Baras & Jobling, 2002). This type of cannibalism was not observed during the present study.

Cannibalistic attacks among fish during the larval and juvenile periods can target the tail, head, or side of the prey (Baras & Jobling, 2002). In early developmental stages of fish, including pike, attacks are usually directed at the tail, but as size differentiation increases attacks directed at the head or from the side occur more frequently (Baras, 1999; Baras & Jobling, 2002; Colchen et al., 2019). Bry et al. (1992) confirm this with observations that 96% of the prey of pike measuring 60–200 mm SL were eaten from the head. Attacking larval pike from the head is a fairly common strategy under rearing conditions, and especially at high densities (Kucharczyk et al., 1997). Similar tendencies were observed in the present study when almost all of the attacks observed during the early

larval stage occurred from the tail, but as the larvae grew, at a size of approximately 17 mm, attacks from side and the head began to appear and were common if the size difference between cannibal and predator was relatively large.

#### Temperature effect on cannibalism intensity

Undoubtedly, larval or juvenile density is a decisive factor in the intensity of cannibalism (Giles et al., 1986). Data from the literature on larval pike of a size range of 18–24 mm TL cultured for 12 days at densities of 9, 44, and 88 larvae·L<sup>-1</sup> indicated there was increased cannibalism intensity at higher densities (Kucharczyk et al., 1997). In the present study, the inverse relationship was observed; cannibalism intensity increased temporally, which corresponded with decreasing larval density from approximately 34.5 to 0.4 n·L<sup>-1</sup> (densities at which the cannibals were collected). However, the decrease in density was accompanied by a simultaneous increase in larval length (from approximately 14 mm to 25 mm). Separating these two factors of density and larval size that shifted temporally is impossible, which prevents fully comparing the current results to those obtained by Kucharczyk et al. (1997). Žiliukienė & Žiliukas (2006) report that the phenomenon of cannibalism in pike measuring from 16–22 mm occurred at both high (30 larvae·L<sup>-1</sup>) and low (2.3 larvae·L<sup>-1</sup>) densities, but they did not analyse the intensity of this phenomenon. Simultaneously, Žiliukienė & Žiliukas (2006) confirm that increased cannibalism intensity occurred with larval growth from 16.4 to 28.2 mm SL, which was also observed in the present study.

Presumably, the observed temperature effect on cannibalism intensity is a consequence of the well-known phenomenon of the positive impact water temperature has on fish metabolism (e.g. Jobling, 1981). Increased metabolism can, in turn, be responsible for increased feeding intensity (e.g. Johnston & Mathias, 1994) and also possibly on the intensity of cannibalism. However, since this subject was not studied in the present research, any attempt to explain the possible mechanisms would be too speculative.

#### Cannibalism and otolith fluctuating asymmetry

FA is considered to be an indicator of developmental instability in organisms (Zakharov, 1992), and as such

it could impact differences in the stress fish experience and in growth rates. FA is reported to be a useful method to test in various fish species the environmental effects of El Niño conditions (Alados et al., 1993), temperature (Lu & Bernatchez, 1999), pollution (Hardersen, 2000), parasitism (Escós et al., 1995; Loot et al., 2001), salinity (Panfili et al., 2005), and fecundity (Hechter et al., 2000). FA is also useful as an indicator of larval fish condition (GrønkJær & Sand, 2003; Somarakis et al., 1997b) and stress (Gagliano et al., 2008; Lemberget & McCornick, 2009; Castillo-Hidalgo et al., 2018; Landaeta et al., 2018), even if the results obtained by Castillo-Hidalgo et al. (2018) were inconsistent. Most recently, Fey et al. (2020) showed for larval trout the significant effect of a magnetic field of 10 mT on otolith FA of larval trout. The opposite result of larval fish growth rate having no effect on FA is also available (Folkvord et al., 2000; Fey & Hare, 2008; Kristoffersen & Magoulas, 2009; Zenteno et al., 2014). Since negative results exist for growth rates and also other indicators (Diaz-Gil, 2015), the usefulness of FA as a fitness and stress indicator is still questioned. In the present study, prey, as opposed to cannibals, represent a group of larvae within a given cohort characterized first of all by slower growth. However, it cannot be excluded that the prey also experience higher stress from being surrounded by larger aggressive individuals. Indeed, for all the otolith size variables the FA index was higher in the present study for prey compared to cannibals, which indicated higher organism instability in the prey. Although these differences were not statistically significant, they can be treated as an indication of the possible effect of differences between cannibals and prey on otolith FA that could be explored in more detail in the future. However, considering the lack of support in the literature for the effect of growth rate differences on FA, the present differences in FA could be related more to the stress experienced than to growth rate differences between cannibals and prey. There is currently no other work that evaluates the FA issue for cannibal/prey groups for comparison.

## Conclusions

Cannibalistic behaviour is a common phenomenon among early life stages of pike, starting as early as 5 dph at a larval size of 13 mm, i.e. as soon as the yolk-

sac is absorbed. The phenomenon is affecting individuals with size difference as small as 0.2 mm, which, for larvae from the size range of 13–19 mm, corresponds to approximately 1.3%. The cannibalism could not occur in low temperature and intensify significantly with increased temperatures. No statistically significant differences in otolith FA between cannibals and prey provided no indication of higher organism instability in the prey.

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## Compliance with ethical standards

**Conflict of interest statement** The authors have no potential conflicts of interest to declare.

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## OŚWIADCZENIE

Współautorzy artykułu pt.: "Positive temperature effects on the initiation and intensity of cannibalistic behaviour of larval pike, *Esox lucius* L. Is cannibalism reflected in otolith fluctuating asymmetry?" opublikowanego w czasopiśmie naukowym Hydrobiologia, <https://doi.org/10.1007/s10750-020-04328-5>, zgodnie oświadczają i podpisani poświadczają, że wkład poszczególnych osób w powstanie publikacji i wynikający z tego procentowy udział był następujący:

### **Martyna Greszkiewicz (80%)**

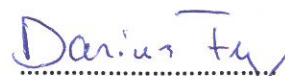
- przygotowanie koncepcji badań
- opracowanie metodyki badań
- wiodący udział w przeprowadzeniu prac eksperymentalnych
- analiza materiału badawczego
- opracowanie danych
- napisanie tekstu i przygotowanie manuskryptu
- wiodący udział w korekcie manuskryptu po recenzjach

### **Dariusz Fey (20%)**

- konsultacje na etapie przygotowania koncepcji oraz metodyki badań
- udział w pracach eksperymentalnych
- konsultacje podczas analizy danych oraz przygotowania manuskryptu
- korekta tekstu przed złożeniem manuskryptu do czasopisma
- udział w korekcie manuskryptu po recenzjach

Każdy ze współautorów oświadcza, że zatwierdził ostateczną wersję artykułu i przyjmuje pełną odpowiedzialność za wszystkie aspekty pracy, zapewniając, że kwestie związane ze starannością i integralnością każdej części pracy zostały odpowiednio zbadane i wyjaśnione.

  
.....  
Martyna Greszkiewicz

  
.....  
Dariusz Fey

## Podsumowanie

Celem naukowym rozprawy doktorskiej pod tytułem "Wpływ wybranych warunków środowiskowych na przeżywalność, tempo wzrostu oraz kanibalizm wczesnych stadiów rozwojowych szczupaka (*Esox lucius*) – badania eksperymentalne" było pogłębienie wiedzy z zakresu ekologii wczesnych stadiów rozwojowych szczupaka (*Esox lucius*), poprzez weryfikację, w warunkach eksperymentalnych, pięciu hipotez badawczych:

**Hipoteza I:** Zasolenie nie wpływa na rozwój embrionalny szczupaka (Publikacja nr 1)

Wykazano, że zasolenie ma istotny wpływ na rozwój embrionalny szczupaka. Zadowalający sukces wylęgu uzyskano jedynie w zasoleniu 0 PSU (89,7%). W 4 PSU obserwowano wylęg, jednak na bardzo niskim poziomie (19,7%). W 7 PSU ikra nie rozwijała się. Sytuacja zmieniła się diametralnie, gdy zapłodnienie wykonano w 0 PSU. Przeniesienie ikry do zasoleń 4 i 7 PSU po 1 godzinie od zapłodnienia, nie miało negatywnego wpływu na rozwój ikry, a sukces wylęgu był wysoki, na poziomie powyżej 80 % w każdym zasoleniu.

**Hipoteza II:** Temperatura nie wpływa na tempo wzrostu larw szczupaka (Publikacja nr 2)

Wykazano, że temperatura wody miała istotny, pozytywny wpływ na tempo wzrostu szczupaka (10°C: 0,25 mm/d; 15°C: 0,48 mm/d; 22°C: 0,83 mm/d). Sam fakt pozytywnego wpływu temperatury na tempo wzrostu larw ryb nie jest wynikiem niespodziewanym, jednak, co istotne, temperatura 22°C nie jest temperaturą przekraczającą optimum dla wzrostu larw i wczesnego narybku szczupaka. Wzrost w wysokiej temperaturze w krótkim czasie (kilkunastu dni) prowadził do pojawienia się dużych różnic wielkości larw z tego samego dnia wylęgu, co może sprzyjać pojawianiu się intensywnego kanibalizmu.

**Hipoteza III:** Temperatura nie wpływa na intensywność kanibalizmu stadiów młodocianych szczupaka (Publikacja nr 3)

Wykazano, że temperatura wody ma istotny wpływ na zjawisko kanibalizmu w okresie larwalnym i wczesno-juwenilnym. W wyższej temperaturze rozpoczęcie ataków kanibalistycznych następuje szybciej, w 22°C już po 5 dniach od wylęgu, a w 15°C po 10 dniach od wylęgu. W 10°C nie zaobserwowano ataków kanibalistycznych. W wyższej temperaturze ataki są również intensywniejsze, zwłaszcza w 22°C. Stosunek wielkości ofiar do wielkości kanibali wahał się średnio od 95% dla ryb 13 mm do 80% dla ryb 27 mm, przy średniej dla wszystkich osobników wynoszącej 87%. Najmniejsze różnice w wielkości między ofiarą a kanibalami (około 0,2 mm) wystąpiły w zakresie wielkości kanibali od 13,4 (różnica = 1,5%) do 18,4 mm (różnica = 1,1%), co znaczy że wielkość ofiary jest praktycznie taka sama, jak wielkość kanibala.

**Hipoteza IV:** Asymetria fluktuacyjna otolitów nie jest bioindykatorem kondycji i sprawności larw szczupaka (Publikacja nr 3)

Wykazano, że nie występuje różnica w asymetrii fluktuacyjnej wielkości otolitów pomiędzy kanibalami a ofiarami, zarówno dla strzałek, jak i kamyczków. Nawet jeśli „bycie ofiarą” jest związane, z definicji, z niższym tempem wzrostu i niższą kondycją, a także funkcjonowaniem w warunkach stresu, nie znalazło to odzwierciedlenia w obliczonym wskaźniku asymetrii fluktuacyjnej.

**Hipoteza V:** Otolity wczesnych stadiów rozwojowych szczupaka nie stanowią skutecznego narzędzia w badaniach ekologii tego gatunku (Publikacja nr 2)

Wykazano, że tempo wzrostu otolitów stadiów młodocianych szczupaka jest ściśle powiązane z ich wzrostem somatycznym – szybciej rosnące osobniki posiadały szybciej rosnące otolity. Oznacza to, że szerokość odkładanych na otolitach przyrostów dobowych jest również powiązana z tempem wzrostu larw i narybku szczupaka. Natomiast zależność wielkość ryby-wielkość otolitu nie była rozregulowana pod wpływem temperatury, co wskazuje na przydatność otolitów szczupaka do wczesnych odczytów tempa wzrostu.

## Wnioski

Skrajnie niski poziom liczebności populacji szczupaka w Zatoce Puckiej rodzi pytania o to, jakie strategie zarządzania należy podjąć. Jak wykazały wyniki naszych badań, zdolność miejscowej populacji, która jest w rzeczywistości słodkowodna, a nie anadromiczna, do rozmnażania się w słonych wodach zatoki jest znikoma. Jednocześnie naturalne tarło szczupaka w słodkiej wodzie na rozlewiskach jest obecnie prawie niemożliwe ze względu na brak takich tarlisk. Zarybianie może być sposobem na poprawę sytuacji, ale tylko na krótką metę, chyba że zostanie opracowany długoterminowy program zarybiania. Jednak długoterminowy program zarybień przeprowadzony na wodach duńskich nie powiódł się, prawdopodobnie z powodu niskiej przeżywalności narybku słodkowodnego, który został wypuszczony (Larsen i in., 2005; Jacobsen i in., 2008) do wód o znacznie wyższym zasoleniu (ok. 8–10 PSU) niż w Zatoce Puckiej (ok. 6–7 PSU). Można więc przypuszczać, że długoterminowy program zarybiania Zatoki Puckiej, wbrew doświadczeniom duńskim, zakończyłby się sukcesem. Warto jednak rozważyć również możliwość przystosowania narybku do słonej wody podczas podchowu, przed wypuszczeniem go jako materiał zarybieniowy.

Najbardziej obiecującym rozwiązaniem wydaje się być połączenie zarybień z przywracaniem tarlisk poprzez wprowadzenie tzw. *Pike Factories*, jak to miało miejsce w Szwecji i Danii (Nilsson i in., 2014; Engstedt i in., 2018). Obecne badanie wykazało, że przeżywalność jaj w wodach zasolonych była dobra, jeśli zostały zapłodnione, a rozwój embrionalny rozpoczął się w słodkiej wodzie; dlatego wprowadzenie fabryk szczupaków może być trafnym rozwiązaniem, nawet na terenach bezpośrednio sąsiadujących z lagunami i zatokami, pomimo ryzyka zalania słonymi wodami. W rzeczywistości zwiększone zasolenie do 7 PSU zaledwie godzinę po zapłodnieniu było korzystne dla powodzenia wylęgu.

Występujące u szczupaka zjawisko kanibalizmu zdecydowanie musi być brane pod uwagę przy próbach odtworzenia populacji tego gatunku. Zachowania kanibalistyczne mogą wystąpić już od 5 dph przy wielkości larwy 13 mm. Różnica wielkości między kanibalem a ofiarą może wynosić zaledwie 0,2 mm, co w przypadku larw z zakresu 13–19 mm wynosi około 1,3%. Według naszych badań intensywność kanibalizmu była silnie uzależniona od temperatury i nasilała się w wyższych temperaturach, a 10°C było

progiem, poniżej którego zjawisko nie występowało. Na terenach zalewowych będących idealnym miejscem tarliskowym dla szczupaka, wiosenne promienie słoneczne ogrzewające wodę mogą zdecydowanie nasilić zjawisko kanibalizmu wśród żerującego narybku tego gatunku. Przywracając miejsca tarliskowe dla tego gatunku należy więc zwrócić uwagę zarówno na dostępność pokarmu dla żerującego narybku szczupaka, jak i, a właściwie przede wszystkim, na zapewnienie odpowiedniej przestrzeni w celu zminimalizowania kanibalizmu. Zwłaszcza, że w wysokich temperaturach tempo wzrostu larw i narybku szczupaka jest szybkie i w krótkim czasie prowadzi do pojawienia się dużych różnic w wielkości pomiędzy osobnikami z tego samego terminu wylęgu.

Planując w przyszłości badania wczesnych stadiów rozwojowych szczupaka w warunkach naturalnych, warto rozważyć możliwość uzyskania niektórych informacji na podstawie analizy mikrostruktury i wielkości otolitów, gdyż okazały się one spełniać podstawowe założenia metodyczne. Nie jest natomiast możliwe zastosowanie analizy asymetrii wielkości otolitów, jako bioindykatora stresu i niższej kondycji larw należących do grupy ofiar, w odniesieniu do kanibali.

Podsumowując, wpływ na sukces odbudowy populacji szczupaka w rejonie Zatoki Puckiej może mieć wiele czynników. Dlatego w celu wypracowania strategii działania kluczowe jest jak najlepsze poznanie, jakie jest oddziaływanie tamtejszego środowiska na wczesne stadia rozwojowe szczupaka, poprzez badania uzupełniające wyniki przedstawione w niniejszej pracy.



Rys. 10. Odtwarzanie tarlisk szczupaka (Pike factories) – Ringsted, Dania.

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