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Efficacy of a novel reproductive tag to index spawn timing

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Abstract

Insight into fish annual reproductive dynamics (including spawn timing) can elucidate key determinants of recruitment. However, few methods, if any, are available to directly index individual spawn timing, hindering study of such processes. To this end, we developed two reproductive tags (RTs; internal and external) and evaluated their performance assessing spawn timing of female yellow perch (*Perca flavescens*). Internal RTs performed well, especially in comparison to external RTs, displaying consistent long-term tag retention and clear tag release synchronized with egg mass release during spawning. These results demonstrate the utility of internal RTs to investigate previously unexplored mechanisms of reproductive timing.

Key words: yellow perch, *Perca flavescens*, reproduction, phenology

Introduction

The spawning phenologies of individual fish may influence population-level annual reproductive success, yet remain poorly understood owing to limited methodologies available to index individual spawn timing. For example, under changing climatic conditions, the plasticity of the relationship between temperature and fish spawning phenology will likely determine a population's adaptive capacity (Thurman et al. 2020). While many studies have demonstrated a concomitant shift to earlier spawning in the face of increasing temperatures (Schneider et al. 2010), precise responses and mechanisms driving such advances remain elusive. Similarly, proposed concepts such as maternal effects (i.e., age- and size-structured transfer of nongenetic advantages from mother to offspring as reviewed by Hixon et al. 2014) may relate to patterns of larger older females spawning earlier and in distinct locations (Trippel et al. 1997). However, many of these theories lack robust methods for linking maternal phenotype to individual effects of spawn timing. In short, research and development into methods allowing accurate and precise indexing of individual spawn timing could contribute to an improved understanding of population-level reproductive dynamics.

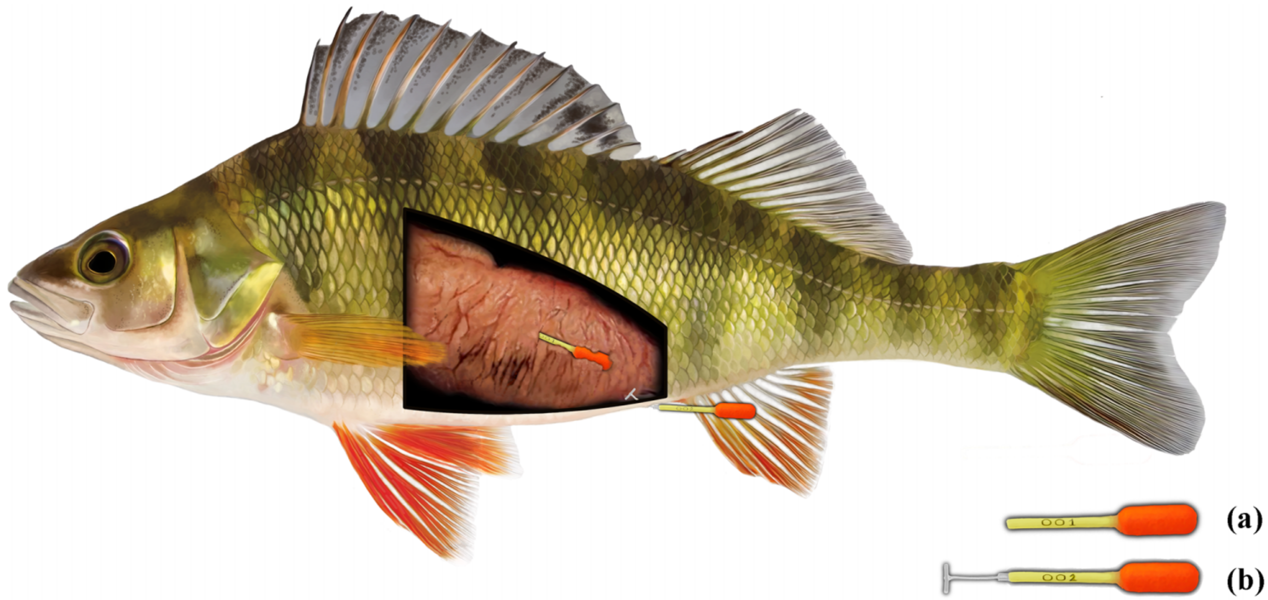
Few studies have investigated the utility of maternal reproductive tags (i.e., tags inserted into gonadal organs; RTs) to index individual spawn timing. The limited number of studies found to experiment with RTs have pursued diverse applications for oviduct-inserted radio and acoustic tags with varying success (Peake et al. 1997; Pierce 2004; Pierce et al. 2007; Skovrind et al. 2013; Binder et al. 2014; Lallement et al. 2022). However, limited evidence that these past tagging methods are robust in assessing natural reproduction in natural and semi-natural systems hinders their use as a reliable index of spawn timing, which remains an invaluable avenue for assessing relationships between reproductive stock structure and early life performance. Due to challenges related to observing spawning in benthic-oriented organisms, we conducted a novel tagging experiment to investigate the utility of RT in indexing spawn timing in female yellow perch (*Perca flavescens*). In a pond experiment, we compared the retention of external to internal RTs (position denoting placement within the reproductive duct) and quantified the proportion of RTs successfully released during the observed spawning season. We then conducted a fully controlled tank experiment to evaluate synchrony between individual spawning events (i.e., release of eggs) and the timing of tag release.

Materials and methods

Study area and species

We evaluated the efficacy of RTs in quantifying female spawning characteristics in a North Carolina-strain (Perquimans River, NC, USA) of yellow perch. The closed population of yellow perch has been maintained at Purdue University's Aquaculture Research Laboratory (ARL) since 1993. Yellow perch were held in a semi-natural, ∼0.1 ha research pond and sustained by feeding upon naturally occurring invertebrate (e.g., aquatic insects and crayfish) and vertebrate (e.g., bullfrogs) prey. Experimental design as detailed below were approved by the Purdue University Institutional Animal Care and Use Committee in protocol no.: 2108002184.

Fig. 1. Demonstrated placement of internal (*a*; 38 x 6.5 mm) or external (*b*; 52 x 6.5 mm) reproductive tags in mature female yellow perch. [Illustration credit: Rene P. Martin, Ph.D.].



Pond assessment: internal vs. external tags

Common across many teleost fish, cystovaries (i.e., continuity between ovary and ovarian duct) in female yellow perch result in the direct expulsion of eggs from the ovary, embedded in a gelatinous, ribbon-like matrix known as the skein (Parker 1942). Direct access to the female ovary permitted manual, non-surgical placement of RTs, either implanted in the ovary (internal) or hung within the ovarian duct (external), in a position set to potentially synchronously release with expulsion of egg skein. Female yellow perch generally spawn during a single event in which the rupture of the urogenital papilla (UGP) and evacuation of egg and skein from the ovary may generate enough force to simultaneously release RTs. Upon its release, a small float would surface the RTs and make it observable and retrievable to document the spawning event. To evaluate the efficacy of external and internal RTs (Fig. 1) in the assessment of spawn timing, a pond experiment was conducted to compare long-term tag retention (>6 months) and recovery.

On 20 October 2021, yellow perch ($n = 60$) were collected from the research pond via seining and transported to an indoor flow-through raceway for processing. Fish were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 75 mg·L^{-1} before being weighed (±0.1 g), measured (±1.0 mm L_T), and injected in the dorsal muscle tissue with a small (~2 × 12 mm), uniquely coded passive integrated transponder (PIT) to facilitate tracking of individual attributes. Sex was visually determined from yellow perch external morphology (Malison et al. 2011). Female maturation status was estimated from biopsy of gonadal tissue obtained via catheter, where the presence of developing eggs was taken to indicate high likelihood of future spawning potential. Female yellow perch displaying high spawning potential (total $n = 35$) were randomly chosen to receive either external ($n = 18$, L_T: range = 223–369 mm, $\bar{x} = 316.9$ mm) or internal RT ($n = 17$, L_T: range = 283–363 mm, $\bar{x} = 335.5$ mm). RTs were fashioned from fluorescent orange cylindrical rig floats (15.875 × 6.25 mm) attached to yellow, Floy extra small (FF-94), T-bar anchor tags (Fig. 1). In fish selected to receive external RTs, the T-bar anchor was deployed in the genital duct using a Mark II Fine Fabric Pistol Grip applicator and blunted needle. When applied, the tag and float remained just outside the UGP. Internal tags were manually inserted into the ovary through the UGP (float end last) after first removing the anchoring T-bar from the RT.

Tagged yellow perch were held in an indoor circular flow-through tank for 1 week to assess rates of short-term tag retention (i.e., proportion of individuals still possessing a RT at the end of 1 week, based on direct recovery of tags from holding tanks) before being returned to the research pond. For individuals that retained their tags during the week in the holding tank, subsequent premature releases of RTs in the experimental pond (i.e., tag recovery before visual confirmation of deposited egg skeins) were documented through weekly visual surveys of pond surface and littoral waters (28 October 2021–March 2022). Weekly surveys were paused at the onset of winter ice (10 December 2021) and resumed (1 February 2022) in advance of ice out. Frequency of monitoring for expelled RTs and egg skeins increased to a daily survey ahead of observed first spring spawning, the duration of each survey ranging from 5 to 23 min (mean = 11 mins). To encourage and concentrate yellow perch spawning to easily accessible littoral areas of the research pond, water levels were lowered from the vegetated edge and woody debris was added to a littoral zone. RTs recovered on or after first observed spawning (22 March 2022) and the end of the experiment (15 April 2022) were recorded as having been successfully released during spawning. Termination of the experiment

Table 1. Number of external ($n = 18$) and internal ($n = 17$) tags recovered during the 2021–2022 pond experiment of yellow perch spawn timing.

Tag recovery	Date range	External (%)	Internal (%)
Short-term release	20–27 October	8 (44.4%)	0
Early release	28 October–21 March	3 (16.7%)	0
Mortality	20 October–15 April	0	4 (23.5%)
Spawned release	22 March–11 April	3 (16.7%)	9 (52.9%)
Manually removed	4 April	2 (11.1%)	NA
Tag not recovered		1 (5.55%)	2 (11.8%)
Fish not recovered		1 (5.55%)	2 (11.8%)

Note: Tag recovery status describes the functional activity of the tag and not the fate of the host (e.g., survival was not tracked post tag release).

was determined from a final sampling event (7 April 2022) that assessed the number of unspent female yellow perch suspected of retaining their RT. Final sampling consisted of multi-pass seining of the entire pond. Observers visually surveyed the research pond for RTs prior to and immediately following sampling to disentangle effects of sampling on tag recovery. Females still retaining RT and suspected of atresia were manually squeezed to confirm absence of egg skein. Externally tagged females that underwent atresia had RTs manually removed.

Tank experiment: evaluating synchrony between spawning and tag release

Results of tag retention and field recovery experiment (2021–2022) supported the use of internal RTs (see Results) but suggested that further evidence of long-term tag retention and synchrony of tag and egg skein expulsion was needed. Therefore, a controlled tank experiment was conducted to assess additional internal tagging uncertainties. On 7 December 2022, a subset of yellow perch ($n = 40$) was seined from a research pond and moved to a flow-through raceway inside ARL. Following previously described methods for assessing spawning potential, a subset of female yellow perch (total $n = 24$; L_T: range = 275–382 mm, $\bar{x} = 340.6$ mm) were tagged (internal RT placement) and randomly assigned to 1 of 12 recirculating tanks (volume = ~189 L tank^{-1}) arranged inside an environmental chamber. In addition, one male yellow perch was added to each tank to stimulate female spawning. Use of environmental chamber permitted fine-scale, automated control over seasonal photoperiod (natural daily phases in nautical twilight at latitude) and thermal conditions (±2 °C) experienced by yellow perch throughout the 160-day experiment (7 December to 16 May). To reduce stress associated with prolonged captive holding and maintain high energetic condition, a fine layer (~5 mm) of pea gravel substrate was added to the bottom of research tanks and live fathead minnows were provided weekly as prey. Water quality parameters (temperature and dissolved oxygen) were monitored daily and maintained through weekly partial water changes (~25%). Individual research tanks were visually assessed each day of the experiment for the presence of RTs and egg skeins. Detected RTs and egg skeins were documented and removed from research tanks to prevent future recounting.

Statistical analysis

The proportion of external and internal RTs successfully recovered from the pond experiment during the observed spawning period (22 March–11 April 2022) were compared in an odds ratio (±95% confidence interval), conditional maximum-likelihood estimate of Fisher's exact test (Fisher 1934, 1962). Linear regression was used to examine synchrony in the release of RT and egg skein documented during tank experiments, where time and date of tag recovery was compared to observed release of skein and eggs. Statistical tests were run in R (version 4.2.2) using *stats* package.

Results

Pond assessment: internal vs. external tags

Internal RTs displayed ideal short-term (1 week) retention (100.0%) in female yellow perch and were absent in all prespawning surveys (Table 1). During the ~3-week observed spawning period, nine internal RTs were recovered in close proximity to added reproductive habitats. In contrast, externally positioned RTs experienced poor short-term retention (55.6%; i.e., 44.4% were lost during initial 1-week observation period) and were occasionally recovered prior to ($n = 3$) and during ($n = 3$) spring spawning (Fig. 2). Odds ratio comparison for the successful recovery of internal to external RTs during spawning (52.94% to 16.67%, respectively; not correcting for pre-spawn mortality) was greater than 1 (odds ratio = 5.331, $P = 0.035$), supporting the use of internal RTs to index individual spawning events. Mortality in a small subset of internally tagged yellow perch (total $n = 4$), prior to the spawning period, confirmed correct positioning of internal RT within the ovary and developing egg skein. The absence of recovered RTs surrounding the 7 April 2022 sampling event indicated that seine sampling likely did not affect tag release and recovery. During this final sampling event, assessment of recaptured yellow perch suggested that atresia had occurred in four females (two external; two internal) and that one external RT was released but unrecovered sometime during the 178-day experiment. Three tagged yellow perch (one external; two

Fig. 2. Cumulative recovery of external and internal reproductive tags (i.e., via premature and spawned releases) throughout the 178-day pond experiment (20 October 2021–15 April 2022).

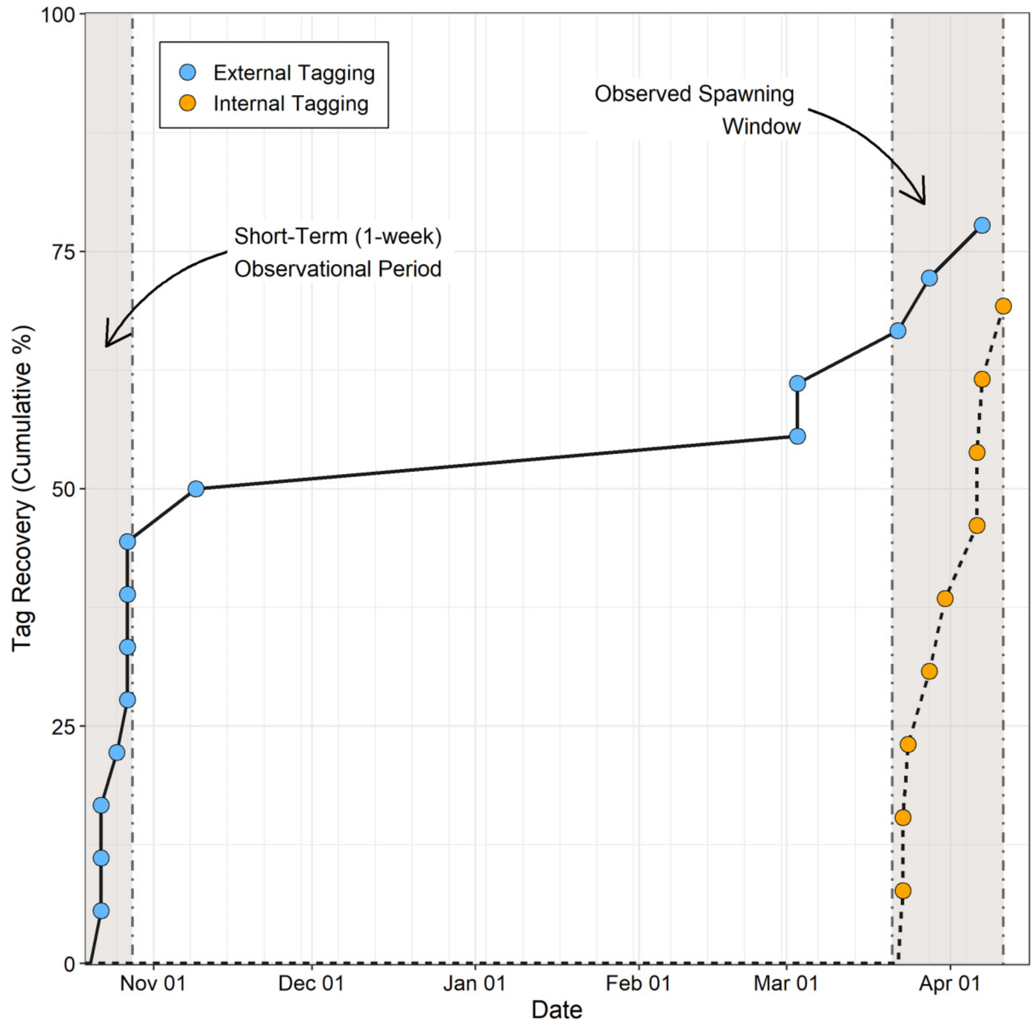


Table 2. Number of internal (n = 24) tags recovered during the 2022–2023 tank experiment of yellow perch spawn timing.

Tag recovery	Date range	Internal (%)
Early release	7 December–16 May	0
Mortality	7 December–16 May	7 (29.2%)
Spawned release	15 March–11 May	17 (70.8%)
Not recovered		0

internal) were not recaptured and were presumed to have succumbed to mortality.

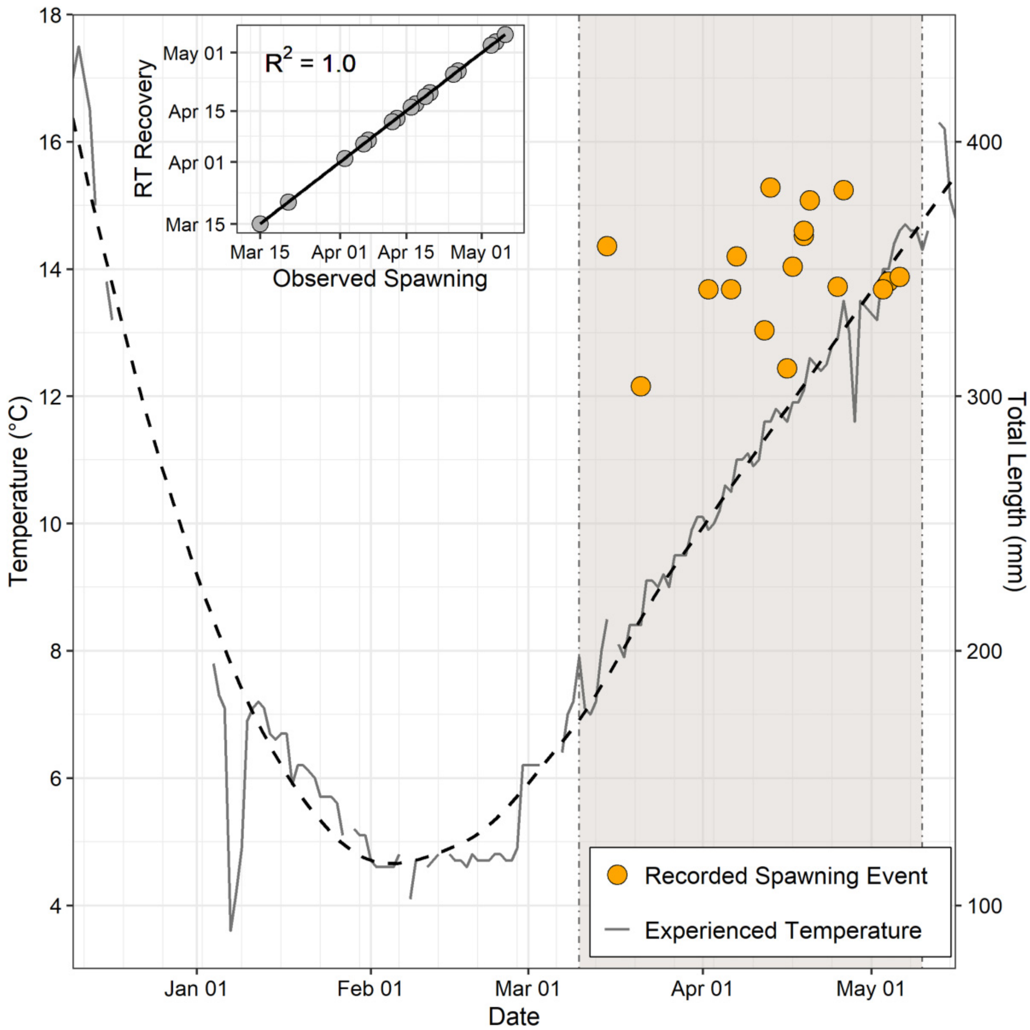
Tank experiment: evaluating synchrony between spawning and tag release

Internal RTs exhibited synchronous release with expelled egg skein ($n = 17$; $R^2 = 1.0$) during the ~8-week spawning period (Table 2; Fig. 3). Spawning in yellow perch was first observed on 15 March 2023 when water temperatures reached 8.5 °C and concluded on 6 May 2023 when temperatures exceeded 14.5 °C. Water temperatures (i.e., 1 March 2023 to 16 May 2023) increased at a rate of 0.124 °C·d^{-1} through the spawning period, best described by the linear relationship: temp = 0.124 × day-of-year − 1.312 ($R^2 = 0.98$). Prior to spring warming, yellow perch experienced moderately cool overwinter temperatures of less than 5 °C for a maximum of 30 days. Seven yellow perch experienced mortality prior to spawning (total = 29%), and five of those mortalities (total = 20.8%) occurred within the first 60 days of the experiment during simulated winter temperatures. Necropsy on perished yellow perch confirmed postmortem tag retention, correct positioning of the tag within the ovary, and developing egg skein, and failed to attribute mortalities directly to internal RTs.

Discussion

Results of these studies demonstrate the utility of internal RTs in indexing individual spawn timing. Internal RTs were well retained within the ovary of female yellow perch, persisting with each individual from time of initial tag placement

Fig. 3. Daily recorded temperature (°C; thin black line) experienced by yellow perch during the 160-day tank experiment (7 December 2022–16 May 2023). Dashed line indicates the fit of local polynomial regression to daily temperature (°C). Orange points depict date of recorded spawning event and maternal length (total length; mm) of internally tagged female yellow perch. Inset figure demonstrates perfect relationship ($R^2 = 1.0$) between the day of reproductive tag (RT) release and observed spawning in tanks.



to its synchronous release with the egg skein during spawning. The recovery of internal RTs in surface waters and clear separation of expelled RTs from egg skein, as documented in controlled tank experiment, conveys additional confidence for use of recovered internal RTs from previous year's pond experiment as an accurate index of female spawning events. These results suggest that consideration be given for the inclusion of internal RTs in future studies attempting to elucidate emergent population-level reproductive dynamics from individual-level reproductive characteristics.

Simplicity of internal RTs and ease at which experiments can be scaled (e.g., larger sample size and longer functional duration) offer several unique advantages for exploring population-level reproductive dynamics. Internal RTs are easily implanted into the female ovary (i.e., usually taking mere seconds) and should theoretically be successfully employed to assess reproductive dynamics in a variety of cystovarians (e.g., most teleost fish). However, future work will need to evaluate the suitability of the RT design in answering study- and species-specific questions, some of which may require tag modification and additional testing. For example, we did not directly assess tagging-mortality versus an untagged control group, and thus future studies may assess impacts of internal RTs on survival.

As currently designed, floating RTs are inexpensive but require a substantial allocation of effort in the form of a daily visual survey that would undoubtably increase with the experiment's spatial extent. Alternatively, more expensive electronic recovery systems (i.e., active and passive tracking) have proven useful in identifying individual spawning locations in larger systems (Pierce 2004; Pierce et al. 2007; Skovrind et al. 2013), but may require paired tagging of fish and ovary to resolve issues detecting spawn timing (as demonstrated in simulations by Binder et al. 2014). Size and battery life of electronic RTs may simultaneously limit applicability to smaller species and those displaying high individual variability in spawn timing. Although manual recovery of floating RTs are well suited to relatively small field and laboratory experiments, we propose that future iterations attempt to integrate advantages of electronic and floating RTs (e.g., small floating radio tag that upon reaching the surface is activated and accurately detected at greater distances) to mitigate investment of effort and expand spatiotemporal monitoring.

Through this RT framework, we might begin by directly assessing current theories regarding the relative importance of reproductive stock structure on population-level reproductive dynamics and its implications to recruitment. Future work to synthesize reproductive dynamics may address questions such as: (1) Do larger fish spawn earlier than smaller conspecifics and does this influence the duration and distribution of emerging larvae? (2) How repeatable are reproductive attributes (e.g., spawn timing and iteroparity) among demographic units of a population? (3) How influential is experienced temperature and seasonal photoperiod to spawning phenology? and (4) How might spawning phenology shift in response to changing climatic conditions, and is there a limit to plasticity of spawn timing? We endorse further research and development into the use of RTs to aid researchers in their assessment of reproductive variation within and among populations.

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Data availability

Data used in this study are provided in full within the published article.

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Competing interests

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