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Title: Population Structure, Age, and Growth

General Information:

The Iowa and Kansas Units are responsible for population structure analysis of the targeted benthic species. Captured specimens will be identified to species either in the field or laboratory. Each Unit will document which identification keys were used for identification. Most captured individuals of species from Table 1 are measured for total length (sturgeon species - fork length) to the nearest mm, and weighed. Length will be measured from the snout to the longest point on the laterally compressed caudal fin. Fish < 1,200-g are weighed with an electronic balance (Ohaus CT1200). Fish that are too long to be effectively weighed by the electronic balance or are > 1200-g are weighed using a 18-kg (40-lb) dial scale (Yamato Accu-Weigh SM-40PK w/ dashpot). Precision for small fish (< 1,200-g) is to the nearest 0.1-g; for large fish (> 1,200-g) precision is to the nearest 50-g. These data are used to evaluate body condition, relative abundance, recruitment, and size structure of the benthic fish population.

Age and growth determinations are also made for 15 of these species (Table 2). Growth rates of individual fish are estimated by aging and back-calculation of lengths at previous ages using specified body structures (Busacker et al. 1990). The body part used for each species, along with the responsible Unit, is shown in Table 2. Scale and spine samples collected from each section will be sent to the appropriate Unit for analysis.

Material & Methods:

A. Ohaus CT1200 electronic balance
   1. Available through Fisher Scientific (Catalog No. 01-920-58) at a cost of about $350 to universities
   2. Accessories available direct from Ohaus (Phone #: 1-800-672-7722)
      a. Calibration weight (~$90), Part No. 76527-02
      b. Hard plastic carrying case (~$40), Part No. 772-56-01.

B. Yamato Accu-Weigh (model SM-40PK) and dashpots can be ordered directly from the company at (719)527-1500 or Yamato Corporation, PO Box 60159, Colorado Springs, CO 80960-0159. Cost for both the scale and dash pot is about $200
   1. Optional: cast aluminum scoop may be appropriate (~$120)

C. Both balances should be calibrated daily following the owner's manuals and using calibrated weights
D. Formalin (10% buffered solution)
E. Whirl-pak (Fisher Scientific - ~$85/500 710-ml bags)
F. Beuhler slow-speed saw (for age and growth only)
G. Thermoplastic Cement (Beuhler Lakeside-70)

Procedure:

I. Length and Weight Measurements
A. For all target benthic fish species, length and weight measurements will be taken.
1. The exception to this is that after the number of individuals needed for age and growth purposes have been preserved for emerald shiner, sand shiner, flathead chub, and Hybognathus spp. the remaining individuals will be enumerated only.
a. Lengths and weights may be taken on these individuals as time permits (Optional).
B. Non-benthic fish will be identified to species and counted.
1. Lengths and weights may also be taken for these individuals as time permits (Optional).

II. Working Tally Sheet
A. A working tally sheet will be used for each segment that is sampled.
B. Body structure samples will be taken from 10 fish per 1-cm length group per segment sampled per field season for "large" fish (river carpsucker, shovelnose sturgeon, smallmouth buffalo, channel catfish, flathead catfish, freshwater drum, sauger, blue sucker).
C. While in the field, a mark will be placed in the appropriate cell of the "working tally sheet" indicating a body structure was taken from that specific length group.
D. This working tally sheet is for **field use only** and will not be recorded in the database.
E. ALL other data collected (e.g. fish number, length, weight, etc.) will be reported on the official data sheet and scale envelopes.
F. For "small" fish (flathead chub, sicklefin chub, emerald shiner, sand shiner, Hybognathus spp.), a maximum of 25 individuals (10 for sicklefin chub) of each species per macrohabitat per segment per field season will be placed in a Whirl-pak, preserved in 10% formalin, and shipped to the appropriate Unit. These batches of specimens should be selected indiscriminately.
1. A completed scale envelop and bar code number should be placed in each whirl-pak to ensure proper identification.
2. Attempts should be made to first preserve specimens that have expired or been injured while sampling.

III. Body Structure Removal
A. Scale Removal
1. Ten or more scales are taken from the scaled fish listed in Table 2.

2. All scales are taken from the area denoted (Table 2).
3. Scale removal will follow procedures as described in Jearld 1983.

B. Spine Removal

1. For channel catfish and flathead catfish, the right pectoral spine is removed following methods by Sneed 1951.
   a. The muscles around the spine should be relaxed to make the spine lie along the body.
   b. Grasp the spine near the base with fingers or pliers and turn counter-clockwise while concurrently pulling outward.
   c. It may be necessary to cut the musculature around the spine for larger individuals.

C. Pectoral Fin and Dorsal Ray Removal

1. The marginal ray of the right pectoral fin from shovelnose sturgeon (Cuerrier 1951) and the first four for blue sucker will be used.
2. The first two dorsal rays will be taken from both river carpsuckers and smallmouth buffalo.
3. The rays are cut parallel and close to the body, while keeping the remaining fin in tact.
4. The severed fin ray is then separated from the attached fin with a knife, scalpel, or scissors.

D. Otolith Removal

1. Otolith removal should follow Jearld (1983) or a similar method.

IV. Sample Storage and Shipping

A. Individual scale, spine, otolith or fin ray samples are placed in separately numbered scale envelopes for later analysis.

B. Extra bar codes for each macrohabitat where specimens were collected and preserved, rather than age structures taken, should be sent with the whirl-pak samples to maintain proper identification of all data when laboratory analysis begins.

C. Specimens shipped to Kansas should be sent to:

Pat Braatan
205 Leasure Hall
Kansas State University
Manhattan, KS 66506

D. Specimens shipped to Iowa should be sent to:

Mark Pegg
Dept. Of Animal Ecology
11 Science II
Iowa State University
Ames, IA 50011

V. Laboratory Preparation of Specimens for Analysis

A. Spine and ray cross-sectioning

1. Most spines and rays are prepared using a Beuhler Low-Speed saw with a 0.0012-in wafering blade. Speed of the saw is set at about mid-range depending on the size of the

specimen to be cut. Care should be taken to sharpen the blade daily and to keep the lubricant relatively free of the saw trimmings that build up after several cuts.

a. **Warning**: When using the slow speed saw, always ensure the piece to be cut is secure. If the piece shifts during the cutting process, **never** attempt to tighten and cut in the same groove. This leads to an increase in the torque on the blade and may cause the blade to break or chip.

2. Soak spine or ray in water for at least 2 hours.
3. With a blunt tool (e.g. tweezers) remove excess flesh from the spine or ray. Do not scrape the structure as this may result in loss of spine/ray tissue.
4. Secure the spine/ray in the saw chuck so that a 90^0 cross-section can be cut. Place a minimal amount of weight on the sawing arm to get a smooth, clean cut.
5. For catfish, the first cut should remove the proximal or "knuckle" of the spine just distal of the basal groove (Sneed 1951).
   a. When the "knuckle" is removed, adjust the saw arm 0.5-mm to make the second cut.
   b. Spines from catfish < 90-mm should be cut with a sharp scalpel by applying firm pressure on the spine. Do not use a "sawing" motion to cut the spine as this will cause splintering. The cross-section will be about 2-mm in thickness but can easily be sanded to a desirable thickness once mounted.
6. Shovelnose sturgeon and smallmouth buffalo rays do not have a basal groove, therefore it is necessary to remove the uneven, splintered proximal portion of the ray.
   a. Three 0.40-mm cross sections are then cut and placed on one glass slide for mounting.
7. Clean excess cutting fluid from the cross section and place the sample on a glass slide for mounting. Place the remainder of the spine/ray back in the scale envelope.

B. Cross section mounting procedures

1. A hot plate should be set at a temperature just warm enough to melt the thermal plastic cement (TPC).
2. Place a small amount of TPC on a glass slide and place the slide on the hot plate.
3. Place the cross sectioned spine or ray in the TPC once the TPC has melted. Leave the slide on the heat source for a few seconds to allow the air bubbles dissipate. It may be helpful to move the spine in a circular motion to facilitate bubble removal.
4. Transfer the slide to a dissecting microscope. While the TPC is still pliable, press the cross section flat against the slide.
5. After the TPC has completely hardened and the spine/ray is in the proper position, file the spine/ray (if necessary) with 1000 or 1200 grit sandpaper to allow light transmission through the cross section. Remove any excess dust and moisture from the slide and remelt the TPC to cover the cross section.
6. Put a strip of adhesive tape on the right hand side of the slide and label it with the appropriate information (i.e. species code, barcode, page #, and ID #).
7. The three cross sections should be mounted from left to right on the slide in the order they were cut.

C. Scale preparation for smallmouth buffalo, blue sucker, and river carpsucker (Scale Press)

1. If scales are dried, remove from scale envelope and soak in water for 1-2 minutes. Remove any regenerated scales.

2. Clean scales using a cloth soaked in 3% KOH.
3. Dab scale on a clean, dry cloth (i.e. Kimwipe) to remove excess water and place on another dry cloth to dry. It usually helps to place a slide and weight on top of the drying scales to prevent the scale edges from curling.
4. When the scales are dry, place them on an acetate slide ridged side down and cover with another acetate slide. Multiple scales from one individual can be placed on one slide, but be sure to leave room on one end of the slide for the marking tape.
5. Feed the covered slide through the scale press. Adjusting the tension properly may require a few trial runs. If the slides curl, the tension is too high; whereas too little tension results in a faint impression.
6. The slide should be marked with tape on one end providing the species code, barcode, page #, and ID #.
7. Pressed scales should be returned to their scale envelope after pressing.

D. Scale preparation for cyprinids (Glass Slides)

1. Remove scales from rows 2,3,4 above the lateral line at the dorsal fin and place them in several drops of water on a glass slide.
2. Choose 10 fairly clean, non-regenerated scales and place them ridge side up in columns of 5 on the left half of the slide.
3. In addition to the 10 uncleaned scales, 10 more must be cleaned and placed on the slide.
4. Scales should be cleaned by lightly brushing them with a dissecting needle. Cleaned scales should be placed in a few drops of water to prevent dessication while the remaining scales are being cleaned. If a scale is torn during this process, it should be discarded and replaced by another scale.
5. Ten cleaned scales should then be placed, ridge side up, in a circle on the right half of the glass slide.
6. While all scales are wet and uncurled, cover with another slide and bind together with adhesive tape (tape should be labeled with species code, barcode, page #, and ID #).
7. To avoid getting scales from different fish on a slide, clean the microscope stage, tweezers, and all other tools before continuing to another specimen.

E. Otolith Preparation

1. Freshwater Drum
   a. Clean the whole otoliths with a cloth or water.
   b. Sand off the anterior portion of the otolith along the dorsal-ventral axis. The anterior portion contains the sulcal matrix which is relatively “cloudy.” Depending on otolith size, sanding starts with 320-600 grit sandpaper to remove the majority of the anterior portion of the otolith. Sanding should proceed to near the junction of the matrix and sulcal groove (essentially at the nucleus). Once the junction is reached, 600 grit sandpaper is used to “polish” the viewing plane.
   c. The posterior end of the otolith is set in a mound of black modeling clay, then the mounted otolith is submerged in immersion oil for reading.
2. Sauger
   a. Clean the whole otolith with a cloth or water.

b. One of the two otoliths is cracked along the dorso-ventral axis through the nucleus. The cracked edge of the posterior half of the otolith is sanded (600 or 1200 grit) for a few strokes to make it smooth.
c. The whole otolith and "cracked" otolith are place in immersion oil and read independently for comparison of the two mounting methods.

3. Flathead Chub and Hybognathus spp.
a. Before mounting otoliths, excessive tissue should be removed with tweezers and dissecting probe.
b. Allow otolith to completely dry before mounting in TPC.
c. Follow the mounting procedures used for spine/ray preparation to mount both otoliths on a glass slide. Mount one otolith on the left side of the slide and the other on the right. Ensure that the entire otolith is covered by the TPC to protect it from breakage.
d. Adhesive tape, labeled with the pertinent information, is placed between the two otoliths.
e. Each otolith is lightly sanded (1200 grit) to expose annuli for age estimation. Care must be taken not to sand through the nucleus.

VI. Estimation of Age and Growth

A. Two readers do the aging.
B. Growth rates of individual fish are estimated by aging and back-calculation of length at age.
1. Growth increments are measured with the assistance of image analysis software.
C. In the laboratory, 10 scales collected from one individual are mounted between on glass or acetate slides
1. Opaque scales are impressed on cellulose acetate slides
2. When assigning ages, all mounted scales are viewed with the exception of regenerated or damaged scales.
3. When determining radii and annular distances, a mean from five randomly selected scales are used.
4. Blue sucker and river carpsucker scales are measured from the focus along a horizontal line to the lateral edge of the scale.
5. Radii and annular measurements are taken from the focus to the longest anterior edge for all other species. Because the anterior portion of flathead chub and Hybognathus spp. scales are compressed and square in nature, this measurement is taken from the focus to the longest "corner."
D. For channel catfish, flathead catfish (Sneed 1951), shovelnose sturgeon (Rossiter et al. 1995), and river carpsuckers, age and growth increments are measured from cross sections previously mounted on glass slides.
1. Radii and annular measurements are taken along the longest possible axis from the origin to the edge of the largest lobe (Marzolf 1955; Jearld 1983).
E. Otolith Measurement
1. Age estimates and annuli distances for freshwater drum are measured and marked along the ventral edge of the sulcal groove beginning at the nucleus.

2. Whole otoliths are used to measure annular and radial distances for all other fish where otoliths are used. These distances are measured from the nucleus to the otolith edge through the longest possible radius.

F. The Fraser-Lee technique is used to back-calculate length at age information based on body structure growth for each species (Busacker et al. 1990).

1. Intercepts (a) for back-calculation are generated from regressions of fish lengths on body structure radius and corrected for size at structure formation.

G. For spines, otoliths, and other body structures that are present at hatch an intercept is not applicable. Therefore, when analyzing these structures other acceptable back-calculation methods may be used (i.e. Dahl-Lea method).

V. Aging Method Validation

A. To validate our aging methods, each structure is independently read by two readers.

B. Specimens are read a second time (by both readers) in instances where the assigned age is not in agreement between the two readers.

C. If discrepancies remain between the two ages after the second reading, both readers will simultaneously view the structure to assign its age.

D. Comparisons also will be made between the two age structures collected for where applicable.

References:

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Table 1. List of target Missouri River benthic fish species used for population structure analysis.

Species	STATUS	COMMERCIAL	SPORT	AGE & GROWTH
Pallid Sturgeon	At Risk			
Shovelnose Sturgeon		x		x
Common Carp		x		
Flathead chub	At Risk			x
Sicklefin Chub	At Risk			x
Sturgeon Chub	At Risk?			
Emerald shiner				x
Sand Shiner				x
Western Silvery Minnow				
Plains Minnow				x
Brassy Minnow				
Fathead Minnow				
Blue Sucker	At Risk			x
Bigmouth Buffalo		x		
Smallmouth Buffalo		x		x
River Carpsucker		x		x
White Sucker				
Shorthead Redhorse		x		
Flathead Catfish		x	x	x
Channel Catfish		x	x	x
Blue Catfish		x	x	
Stonecat				
Burbot	At Risk?			
Sauger			x	x
Walleye			x	
Freshwater Drum		x	x	x

Table 2. Missouri River benthic fish species to be used for age and growth analysis. The Unit column denotes the unit (Iowa State - IA; Kansas State - KS) where body structures are sent for analysis.

Species	Structure	Location	Protocol	Ship To
River Carpsucker	Scales Rays	Rows 2,3,4 above lateral line below dorsal origin 1st & 2nd dorsal fin rays	10/cm group/segment	KS
Freshwater Drum	Scales Otoliths	Posterior edge of pectoral fin	10/cm group/segment	KS
Sauger	Scales Otoliths	Posterior edge of pectoral fin	10/cm group/segment	KS
Blue Sucker	Scales Rays	Rows 2,3,4 above lateral line below dorsal origin 1st four right pectoral fin rays	10/cm group/segment	KS
Emerald Shiner	25 per macrohabitat per segment - preserved			KS
Sand Shiner	25 per macrohabitat per segment - preserved			KS
Sicklefin Chub	10 per macrohabitat per segment - preserved			KS
Brassy Minnow	25 per macrohabitat per segment - preserved			IA
Plains Minnow	25 per macrohabitat per segment - preserved			IA
W. Silvery Minnow	25 per macrohabitat per segment - preserved			IA
Flathead Chub	25 per macrohabitat per segment - preserved			IA
Smallmouth Buffalo	Scales Rays	Rows 2,3,4 above lateral line below dorsal origin 1st & 2nd dorsal fin rays	10/cm group/segment	IA
Channel Catfish	Spines	Right pectoral spine	10/cm group/segment	IA
Flathead Catfish	Spines	Right pectoral spine	10/cm group/segment	KS
Shovelnose Sturgeon	Rays	Right pectoral fin ray	10/cm group/segment	IA

TALLY SHEET FOR STRUCTURE SAMPLES PER SEGMENT

SEGMENT _____

TL/FL	FWDM	RVCS	SNSG	SGER	SMBF	BUSK	CNCF	FHCF	TL/FL	FWDM	RVCS	SNSG	SGER	SMBF	BUSK	CNCF	FHCF
30-39									470-								
40-49									480-								
50-59									490-								
60-69									500-								
70-79									510-								
80-89									520-								
90-99									530-								
100-									540-								
110-									550-								
120-									560-								
130-									570-								
140-									580-								
150-									590-								
160-									600-								
170-									610-								
180-									620-								
190-									630-								
200-									640-								
210-									650-								
220-									660-								
230-									670-								
240-									680-								
250-									690-								
260-									700-								
270-									710-								
280-									720-								
290-									730-								
300-									740-								
310-									750-								
320-									760-								
330-									770-								
340-									780-								
350-									790-								
360-									800-								
370-									810-								
380-									820-								
390-									830-								
400-									840-								
410-									850-								
420-									860-								
430-									870-								
440-									880-								
450-									890-								
460-									900-								