



Florida Department of Environmental Protection

A COMPARISON BETWEEN NOTOX AND FORMALIN PRESERVATION EFFECTIVENESS IN FRESHWATER BENTHIC MACROINVERTEBRATE SAMPLES

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Purpose of Study

- Florida Department of Environmental Protection (FDEP) received a request to use NOTOXhisto[®] (NoTox), a non-formalin-based fixative, for preserving macroinvertebrate samples
 - FDEP SOPs require formalin for macroinvertebrate preservation
 - Concerns about toxicity and cost of disposal for formalin prompted interest of stakeholder
- Stakeholder study: found NoTox to be comparable to formalin in effectiveness
- FDEP wanted to expand on the scope of the original study to compare Notox and Formalin:
 - in a variety of taxonomic groups
 - over longer holding times
 - in the presence of detritus



Methods

- Macroinvertebrates were collected with No. 30 (approx. 600 micron) mesh dipnets, from 3 streams in FL's Big Bend region: Yon Creek (5/26/15), Mule Creek (5/29/15), and McBride Slough (6/17/15).
- Organisms were removed from detritus, brought to lab in jars of site water, and preserved in either formalin or Notox
- Identical jars were made for each preservative, holding the same numbers of individuals for each taxonomic group (order), for a paired comparison
- Individuals from these containers were examined at 1 week, 1 month, 3 months (approx.), and 6 months (approx.) post-sampling, and identified to the lowest practical taxonomic level



GEORGIA

Ochlocknee

319

5 ft

Chipola River

GADSDEN

Yon Creek

Mufe Creek

I-10-E

263

LEON

I-10-W

CALHOUN

Lake Tynguin

Tallahassee

WAKULLA

Apalachicola National Forest

McBride Slough

319

Coastal Hwy

98

GULF

FRANKLIN



Metrics

Metrics observed for each specimen:

- **Color:** Normal (N) or Abnormal (A)
- **Tissue Condition:** Disintegrating/Brittle (1), Soft (2), Firm (3)
- **Body Integrity:** Poor (1), Acceptable (2), and Very Good (3)
- **Identifiability:** Cannot ID (1), Difficult to ID (2), and Easy to ID (3)



Color

- Color differences between Formalin preserved animals and NoTox preserved animals were mostly unremarkable
- Decapods were most likely to change color when preserved, and they often changed color in both preservatives
 - They usually turned a darker orange in NoTox samples
 - This color change did not affect The taxonomists' ability to identify the animals



Color comparison between crayfish. NoTox (Top) and Formalin (Bottom)



Tissue Condition, all holding times (1=disintegrating/brittle; 2=soft; 3=firm)

Taxa	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
AMPHIPODA	104	3.00000	115	2.94783	0.0981
COLEOPTERA	372	2.96774	376	2.98138	0.2829
DECAPODA	154	2.88961	162	2.89506	0.8869
DIPTERA	105	2.69524	109	2.89908	0.0003
EPHEMEROPTERA	210	2.49524	182	2.79670	<0.0001
GASTROPODA	297	3.00000	340	2.98824	0.0609
HETEROPTERA	150	3.00000	166	3.00000	---
ODONATA	173	2.82081	176	2.83523	0.7273
PLECOPTERA	175	2.94857	172	2.98837	0.0344
TRICHOPTERA	156	2.92949	152	2.97368	0.0720



Body Integrity, all holding times (Poor=1, Acceptable=2, Very Good =3)

Taxa	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value ($p > 0.05$ no significant difference)
AMPHIPODA	104	2.91346	115	2.93043	0.6918
COLEOPTERA	372	2.95968	376	2.97606	0.2734
DECAPODA	154	2.83766	162	2.88889	0.1971
DIPTERA	105	2.76190	109	2.91743	0.0034
EPHEMEROPTERA	210	2.34762	182	2.57692	0.0001
GASTROPODA	297	2.99327	340	2.98824	0.5713
HETEROPTERA	150	3.00000	166	2.97590	0.1198
ODONATA	173	2.86705	176	2.84091	0.5489
PLECOPTERA	175	2.77143	172	2.95930	<0.0001
TRICHOPTERA	156	2.96154	152	2.98684	0.1638



Identifiability, all holding times (1=Cannot ID; 2=Difficult but doable; 3=Easy to ID)

Taxa	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
AMPHIPODA	104	3.00000	115	2.96522	0.1193
COLEOPTERA	372	2.99194	376	3.00000	0.0812
DECAPODA	154	3.00000	162	3.00000	---
DIPTERA	105	2.99048	109	2.98165	0.5851
EPHEMEROPTERA	210	2.77143	182	2.90659	0.0016
GASTROPODA	297	3.00000	340	3.00000	---
HETEROPTERA	150	3.00000	166	3.00000	---
ODONATA	173	2.99422	176	2.98864	0.5734
PLECOPTERA	175	3.00000	172	2.98837	0.1534
TRICHOPTERA	156	3.00000	152	2.99342	0.3118



Ephemeroptera Tissue Condition, Body Integrity and Identifiability, at various holding times

Tissue Condition					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	53	2.52830	49	2.79592	0.0110
28 Days	51	2.49020	39	2.82051	0.0028
84 Days	52	2.50000	48	2.79167	0.0033
168 days	54	2.46296	46	2.78261	0.0032
Body Integrity					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	53	2.22642	49	2.32653	0.3888
28 Days	51	2.31373	39	2.58974	0.0201
84 Days	52	2.38462	48	2.66667	0.0203
168 days	54	2.46296	46	2.73913	0.0220
Identifiability					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	53	2.69811	49	2.83673	0.1721
28 Days	51	2.66667	39	2.92308	0.0183
84 Days	52	2.78846	48	2.93750	0.0320
168 days	54	2.92593	46	2.93478	0.8643



Diptera Tissue Condition, at various holding times

Tissue Condition					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	24	2.79167	26	2.92308	0.1882
28 Days	25	2.88000	30	2.90000	0.8434
84 Days	29	2.62069	26	2.88462	0.0248
168 days	27	2.51852	27	2.88889	0.0023

Body Integrity					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	24	2.83333	26	2.92308	0.3393
28 Days	25	2.76000	30	2.90000	0.2671
84 Days	29	2.72414	26	2.92308	0.0577
168 days	27	2.74074	27	2.92593	0.0701

Identifiability					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	24	3.00000	26	2.96154	0.3419
28 Days	25	3.00000	30	2.96667	0.3662
84 Days	29	3.00000	26	3.00000	----
168 days	27	2.96296	27	3.00000	0.3219



Plecoptera Body Integrity, at various holding times

Tissue Condition					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	44	2.95455	35	2.94286	0.8168
28 Days	45	2.95556	46	3.00000	0.1515
84 Days	43	2.95349	45	3.00000	0.1467
168 days	43	2.93023	46	3.00000	0.0697
Body Integrity					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	44	2.86364	35	2.94286	0.2519
28 Days	45	2.95556	46	2.97826	0.5494
84 Days	43	2.65116	45	2.93333	0.0055
168 days	43	2.60465	46	2.97826	0.0003
Identifiability					
Holding Time	Formalin number	Formalin mean	Notox number	Notox mean	ANOVA p value (p>0.05 no significant difference)
7 Days	44	3.00000	35	2.94286	0.1110
28 Days	45	3.00000	46	3.00000	---
84 Days	43	3.00000	45	3.00000	---
168 days	43	3.00000	46	3.00000	---



Conclusions, so far

- Both preservatives performed well, with a high percentage of specimens with firm tissue condition, very good body integrity, and easy to identify
- Tissue condition and body integrity with Notox was significantly better for Diptera, Ephemeroptera, and Plecoptera, for all holding times
- Identifiability with Notox was significantly better for Ephemeroptera, for all holding times
- In looking specifically at various holding times, only Ephemeroptera had better results in Notox for all metrics
- Have SCI data (2 reps X 2 sites) to analyze, to see if there is difference in presence of detritus.



Practical Concerns

- The price of NoTox is ~\$55/gallon* while formalin is ~\$8.50/gallon*
- NoTox is non-carcinogenic but, because it is flammable, FDEP cannot dispose of it down the drain as the manufacturer suggests, making disposal cost the same as formalin, which must be disposed of as a hazardous waste (approx. \$7.25 per gallon)
- FDEP recycles formalin, making overall cost much lower (purchase and disposal costs reduced)
- FDEP Laboratory has never exceeded the OSHA permissible exposure limits
- Other entities may be subject to different regulations for disposal

* Prices from Fisher Scientific in March, 2016.



Questions or comments?

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